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(54) Title: CONFORMATIONALLY RESTRICTED POLYAMINE ANALOGS AS DISEASE THERAPIES			
(57) Abstract			
<p>Novel conformationally restricted polyamine analogs are provided, as well as compositions comprising these novel polyamine analogs. Methods of using the novel polyamine analogs in treatment of diseases such as cancer are also provided. Also provided is a method of delivering these analogs specifically to tumor cells by covalently attaching polyamine analogs to porphyrin compounds, along with novel polyamine-porphyrin covalent conjugates.</p>			

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CONFORMATIONALLY RESTRICTED POLYAMINE ANALOGS AS DISEASE THERAPIES

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims priority benefit of co-pending provisional patent application U.S. Serial No. 60/131,779, filed on April 30, 1999. The content of that application is hereby incorporated by reference herein in its entirety.

STATEMENT OF RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH

10 Not applicable.

TECHNICAL FIELD

This invention relates to conformationally restricted polyamine analogs, and to
15 porphyrin-polyamine conjugates. This invention also relates to uses of these analogs or conjugates in various medicinal applications, including suppressing cell proliferation and treating various diseases, including cancer.

BACKGROUND OF THE INVENTION

20 Natural polyamines, e.g., spermidine, norspermidine, homospermidine, 1,4-diaminobutane (putrescine), and spermine, are simple aliphatic amines produced in eukaryotic cells by a highly regulated metabolic apparatus. Polyamine levels and the activity of the polyamine biosynthetic apparatus tend to be high in dividing mammalian cells and low in quiescent cells. Populations of cells depleted of their polyamine content
25 stop growing and may die. Janne et al. (1978) *A. Biochim. Biophys. Acta.* 473:241 and Pegg et al. (1982) *Am. J. Cell. Physiol.* 243:212-221. Polyamines are reviewed in Morgan (1998) *Methods. Mol. Biol.* 79:3-30.

Several lines of evidence indicate that polyamines, particularly spermidine, are required for cell proliferation: (i) they are found in greater amounts in growing than in non-
30 growing tissues; (ii) prokaryotic and eukaryotic mutants deficient in polyamine biosynthesis are auxotrophic for polyamines; and (iii) inhibitors specific for polyamine biosynthesis also inhibit cell growth. Despite this evidence, the precise biological role of

polyamines in cell proliferation is uncertain. It has been suggested that polyamines, by virtue of their charged nature under physiological conditions and their conformational flexibility, might serve to stabilize macromolecules, such as nucleic acids, by anion neutralization. Hafner et al. (1979) *J. Biol. Chem.* 254:12419; Pohjatipelto et al. (1981) 5 *Nature* 293:475; Mamont et al. (1978) *Biochem. Biophys. Res. Commun.* 81:58; Bloomfield et al. (1981) in *Polyamines in Biology and Medicine*, Morris et al., Eds., Dekker, New York, pp. 183-205.

A treatment approach has been devised based on the observation that increases in the polyamine pool suppress polyamine biosynthesis. Porter et al. (1988) in *Advances in Enzyme Regulation*, Pergamon Press, pp. 57-79. This approach attempts to identify 10 polyamine analogs which down-regulate polyamine biosynthesis, but which do not perform the polyamine functions required for cell growth. BESPM, a N-bis(ethyl) analog of spermine, has served as a model compound for this strategy. BESPM rapidly suppresses polyamine biosynthetic enzymes, depletes natural polyamine pools, and inhibits cell growth 15 *in vitro*. Porter et al. (1987) *Cancer Res.* 47:2821-2825. In addition, BESPM suppresses polyamine uptake (Byers et al. (1990) *J. Physiol.* 142:460-467; and Kramer et al. (1993) *J. Cell. Physiol.* 115:399-407), and thus minimizes the ability of tumor cells to meet their polyamine requirement by taking them up from their environment. BESPM and related 20 analogs also induce the polyamine metabolizing enzyme spermidine/spermine N¹-acetyltransferase (SSAT) in certain human carcinoma cell lines.

BESPM and other polyamine analogs have been used, or proposed for use, in treating a large variety of diseases, including a number of different cancers. U.S. Patent No. 5,541,230. Polyamine analogs demonstrated, for example, potent antitumor activity against several melanoma cell lines and tumors *in vitro* (Porter et al. (1991) *Cancer Res.* 51:3715-25 3720; Shappell et al. (1992) *Anticancer Res.* 12:1083-1090) and *in vivo* using tumors growing as xenografts in athymic mice (Bernacki et al. (1992) *Cancer Res.* 52:2424-2430; Porter et al. (1993) *Cancer Res.* 53:581-586). Potent antitumor activity of bis-ethyl 30 spermine analogs has also been demonstrated for pancreatic cancer cell lines *in vitro* (Chang et al. (1992) *Cancer Chemother. Pharmacol.* 30:183-188) and *in vivo* (Chang et al. (1992) *Cancer Chemother. Pharmacol.* 30:179-182). Polyamine analogs have also been suggested for use in treating brain tumor therapy. Redgate et al. (1995) *J. Neurooncol.* 25:167-79. In addition to being useful against cancers of the brain, pancreas, and skin,

polyamine analogs are also useful against cancers of the bladder, bone, breast, colon, digestive tract, lung and ovaries. Chang et al. (1993) *J. Urol.* 150:1293-7; Snyder et al. (1994) *Anticancer Res.* 14:347-56; Yuan et al. (1994) *Biochem. Pharmacol.* 47:1587-92; Davidson et al. (1993) *Cancer Res.* 53:2071-5; Berchtold et al. (1998) *J. Cell. Physiol.* 174:380-6; Porter et al. (1988) *Adv. Exp. Med. Biol.* 250:677-90; U.S. Patent Nos. 5,498,522 and 5,374,658. U.S. Patent No. 5,498,522 presents the use of spermidine/spermine N¹-acetyltransferase as a prognostic indicator of the efficacy of a polyamine analog against a malignant tumor.

5 Polyamine analogs have been used to treat cancer of the prostate. Mi et al. (1988) *Prostate* 34:51-60. Polyamines are produced in large amounts by the prostate gland and are abundant in the seminal fluid. Herr et al. (1984) *Cancer* 53:1294-8. Polyamine analogs such as BE-4444, BE-373, and BE-333 are particularly effective in inhibiting prostate xenograft tumors in nude mice. Zagaja et al. (1998) *Cancer Chem. Pharm.* 41:505-512; Jeffers et al. (1997) *Cancer Chem. Pharm.* 40:172-179; Feuerstein et al. (1991) *J. Cell. Biochem.* 46:37-47; and Marton et al. (1995) *Ann. Rev. Pharm. Toxicol.* 35:55-91.

10 In addition to treating cancer, polyamines and their analogs have uses in treating a number of other diseases and in numerous other medicinal applications. Oxidized polyamines are believed to inhibit growth of parasites (Morgan et al. (1983) *Adv. Polyamine Res.* 4: 169-174; Morgan et al. (1986) *Biochem. J.* 236:97-101; and U.S. Patent No. 4,935,449) and suppress infectivity of selected strains of bacteria and fungi (Bachrach et al. (1971) *J. Gen. Virol.* 13:415-22; Nishimura et al. (1971) *Biochim. Biophys. Acta* 247:153-6; and U.S. Patent No. 5,744,453). Polyamines such as spermine and polyamine analogs are also anti-viral and some are anti-insecticidal. Bachrach et al. (1972) *Appl. Microbiol.* 23:232-5; Bachrach et al. (1971) *J. Gen. Virol.* 11:1-9; U.S. Patent Nos. 5,021,409; 5,606,053; 5,608,061; 5,612,478; and 5,681,837. In addition, oxidized polyamines, such as spermine dialdehyde, for example, can be used in treatment of tissue grafts and other organs for transplantation. U.S. Patent No. 5,374,658. Polyamine analogs can also be used to treat neurodegenerative diseases and neurotrauma such as stroke. U.S. Patent Nos. 5,646,188 and 5,677,349. Polyamine analogs have also been reported to be 15 useful as anti-psoratic agents, and in the treatment of epilepsy, Alzheimer's disease, and multiple sclerosis, as described in U.S. Patent No. 5,646,188. Polyamine analogs are also useful in treating and preventing restenosis. U.S. Patent No. 5,516,807. Polyamine analogs

are also useful in treatment of gastric ulcers. Igarashi et al. (1990) *Biochem. Biophys. Res. Commun.* 172:715-20. In addition, polyamine derivatives including N-alkythio polyamine derivatives, polyamine thiols, and polyamine phenols are useful as radioprotective agents for normal tissues during radiotherapy. U.S. Patent Nos. 5,217,964; 5,354,782; and
5 5,434,145.

Polyamines and their analogs can be administered alone or in conjunction with additional agents. For example, therapeutic polyamines can be administered along with 1,3-bis (2-chloroethyl)-1-nitrosourea. U.S. Patent No. 5,541,230. In treating cancer, polyamines can be co-administered with various cytotoxic agents, including antineoplastic
10 vinca alkaloids, antibiotics, antimetabolites, and platinum coordination complexes. U.S. Patent No. 5,654,287.

In addition to the various aforementioned medicinal uses, polyamines and polyamine analogs have a variety of industrial uses, including derivatization of silica. U.S. Patent No. 5,763,388. Polyamines have also been used in conjunction with other
15 clarification aids to treat wastewaters. U.S. Patent Nos. 5,413,719 and 5,707,532. The combination of aluminum chlorohydrate and a polyamine is an effective emulsion breaker for reverse (oil-in-water) emulsions, e.g. in a matrix comprising mostly oil as encountered in a crude oil desalter unit. U.S. Patent No. 5,607,574. Polyamines are also useful in deodorizing polysulfides. U.S. S.I.R. H1,633. Polyamines are also used in industrial dyes.
20 U.S. Patent No. 5,672,202. Polyamines and hot water can also be used in manufacturing microcapsules. U.S. Patent No. 5,401,443. The antioxidative and metal-chelating effects of polyamines are reviewed in Lovaas (1997) *Adv. Pharmacol.* 38:119-149.

It would be advantageous to develop novel polyamine analogs for various uses, including disease treatment.

25 All references cited herein are hereby incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

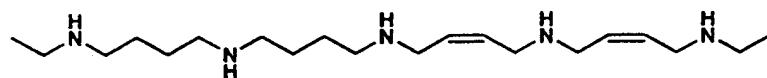
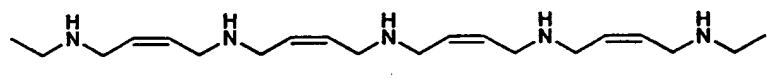
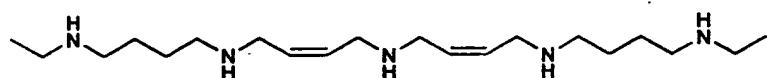
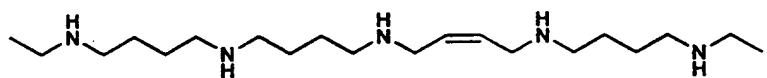
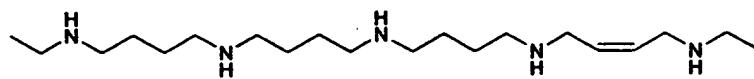
The invention provides novel polyamine analogs, compositions comprising a polyamine analog, and methods using the analogs and compositions. In one embodiment, the polyamine analog is conformationally restricted.
30

In another embodiment, the polyamine analog is selected from among compounds of the formula:

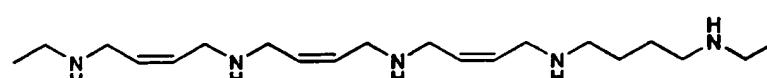
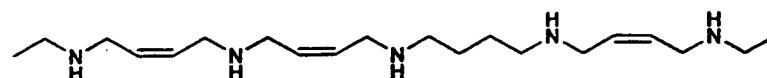
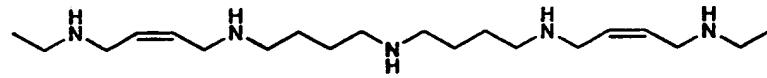
E-NH-B-A-B-NH-B-A-B-NH-B-A-B-NH-B-A-B-NH-E

where A is independently selected from the group consisting of a single bond, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl; B is independently selected from the group consisting of: a single bond, C₁-C₆ alkyl, and

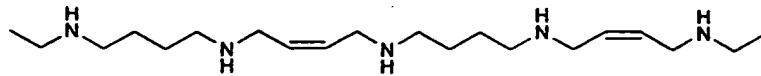
5 C₂-C₆ alkenyl; and E is independently selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl; with the proviso that either at least one A moiety is selected from the group consisting of C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl, or at least one B moiety is selected from the group consisting of C₂-C₆ alkenyl; and all salts and stereoisomers thereof. Specific embodiments of compounds of this type include



15



, and



, and all salts and

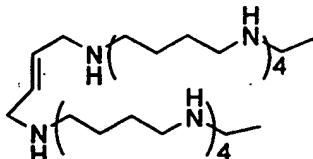
stereoisomers thereof.

In another embodiment, the polyamine analog is selected from among the group of compounds of the formula:

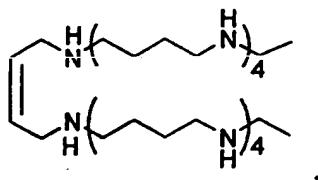
5 E-NH-B-A-B-NH-B-A-B-NH-B-A-B-NH(-B-A-B-NH)_x-E

wherein A is independently selected from the group consisting of a single bond, C₆-C₂ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl; B is independently selected from the group consisting of a single bond, C₁-C₆ alkyl, and C₂-C₆ alkenyl; E is independently selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl; and x is an integer from 2 to 16; with the proviso that either at least one A moiety is selected from the group consisting of C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl, or at least one B moiety is selected from the group consisting of C₂-C₆ alkenyl; and all salts and stereoisomers thereof.

10 15 Specific embodiments of compounds of this type include



and



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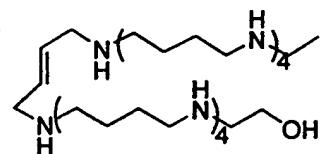
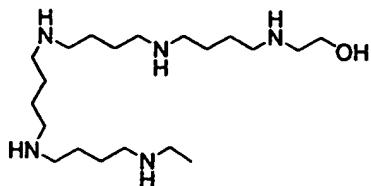
and all salts and stereoisomers thereof.

20 In another embodiment, the polyamine analog is selected from among the group of compounds of the formula

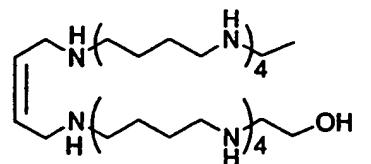
E-NH-B-A-B-NH-B-A-B-NH-B-A-B-NH(-B-A-B-NH)_x-E

wherein A is independently selected from the group consisting of a single bond, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl; B is independently selected from the group consisting of a single bond, C₁-C₆ alkyl, and C₂-C₆ alkenyl; E is independently selected from the group consisting of C₁-C₆ alkyl, C₁-C₆ alkanol, C₃-C₆ cycloalkanol, and C₃-C₆ hydroxyaryl, with the proviso that at least one E moiety be selected from the group consisting of C₁-C₆ alkanol, C₃-C₆ cycloalkanol, and C₃-C₆ hydroxyaryl; and x is an integer from 0 to 16; and all salts or stereoisomers thereof.

5 Specific embodiments of compounds of this type include



10 , and



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and all salts and stereoisomers thereof.

In another embodiment, the polyamine analog is selected from among the group of 15 compounds of the formula



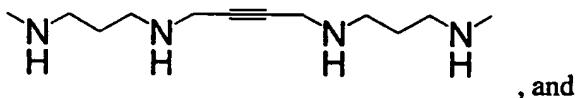
wherein A is selected from the group consisting of C₂-C₆ alkynyl; B is independently selected from the group consisting of: a single bond, C₁-C₆ alkyl, and C₂-C₆ alkenyl; D is independently selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloalkenyl, and C₃-C₆ cycloaryl; and E is independently selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆

20 alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloalkenyl, and C₃-C₆ cycloaryl; and E is independently selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆

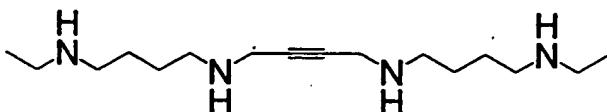
cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl; and all salts and stereoisomers thereof. Specific embodiments of compounds of this type include



5



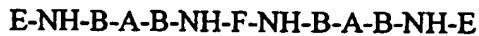
, and



;

10 and all salts and stereoisomers thereof.

In another embodiment, the polyamine analog is selected from among the group of compounds of the formula



wherein F is selected from the group consisting of C₁-C₆ alkyl; A is independently selected

15 from the group consisting of: a single bond, C₁-C₆ alkyl; C₂-C₆ alkenyl, C₂-C₆ alkynyl,

C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl; B is independently selected

from the group consisting of: a single bond, C₁-C₆ alkyl, and C₂-C₆ alkenyl; and E is

independently selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆

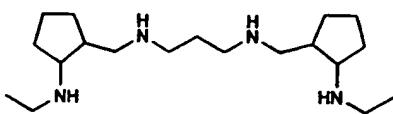
alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl; with the proviso that

20 either at least one A moiety is selected from the group consisting of C₂-C₆ alkenyl, C₂-C₆

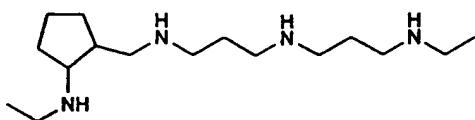
alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl, or at least one B

moiety is selected from the group consisting of C₂-C₆ alkenyl; and all salts and

stereoisomers thereof. Specific embodiments of compounds of this type include

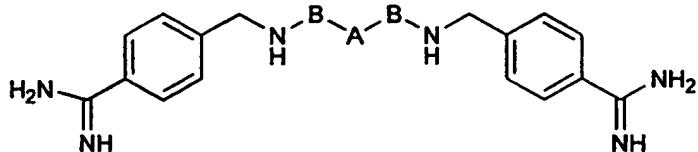


and



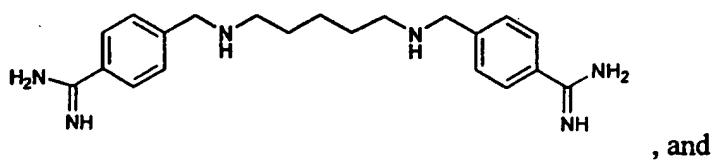
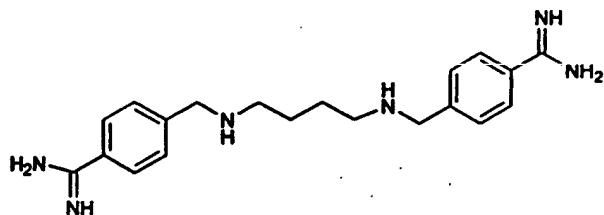
and all salts and stereoisomers thereof.

5 In another embodiment, the polyamine analog is selected from among the group of compounds of the formula

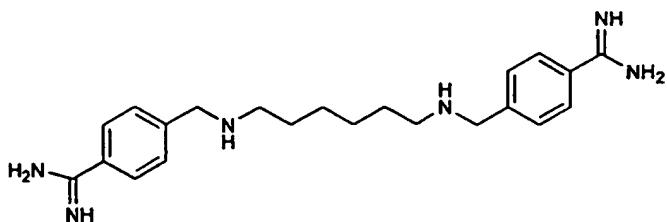


10 wherein A is independently selected from the group consisting of a single bond, C₁-C₆ alkyl; C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl; B is independently selected from the group consisting of: a single bond, C₁-C₆ alkyl, and C₂-C₆ alkenyl; with the proviso that A and both B moieties are not all a single bond; and all salts and stereoisomers thereof. Specific embodiments of compounds of this type include

15



, and



and all salts and stereoisomers thereof.

In another embodiment, the polyamine analog is selected from among the group of compounds of the formula

E-NH-D-NH-B-A-B-NH-D-NH-E

wherein A is selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆

alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloalkenyl, and C₃-C₆ cycloaryl; B is independently selected from the group consisting of: a single bond, C₁-C₆ alkyl, and C₂-C₆ alkenyl; D is

independently selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆

alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloalkenyl, and C₃-C₆ cycloaryl; and E is independently selected from the group consisting of H and C₁-C₆ alkyl; with the proviso that either at least

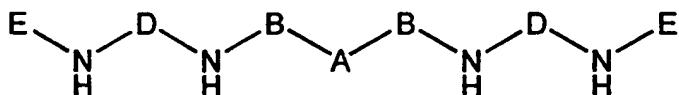
one A moiety is selected from the group consisting of C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl, or at least one B moiety is selected

from the group consisting of C₂-C₆ alkenyl; and the proviso that at least one E is selected from the group consisting of H or methyl; and all salts and stereoisomers thereof. A specific embodiment of compounds of this type includes



and all salts and stereoisomers thereof.

In another embodiment, the polyamine analog is selected from among the group of compounds of the formula

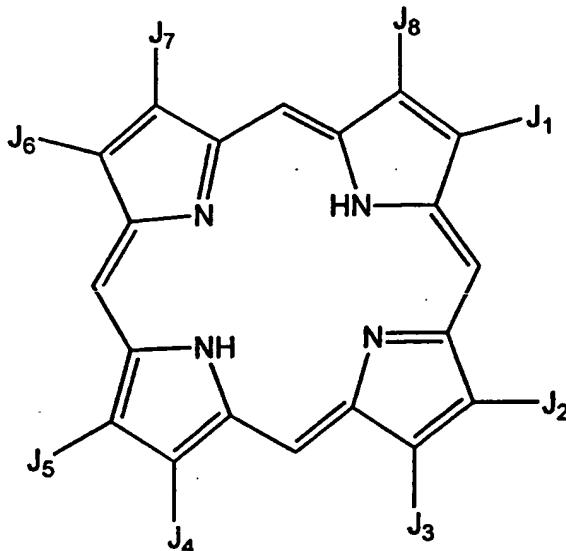


wherein A is *trans*-ethene (no stereochemical variation about the double bond being permitted, that is, *cis*-ethene is specifically excluded); B is independently selected from the

group consisting of: a single bond, C₁-C₆ alkyl, and C₂-C₆ alkenyl; D is independently selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl, with the proviso that D is not C₃ alkyl; and E is independently selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl; and all salts thereof.

In another embodiment the invention also provides conjugates of polyamines to porphyrin compounds, where the polyamines are linked via a covalent bond to the porphyrin compound. Polyamines useful for this embodiment of the invention include, but are not limited to, the polyamines described in the previous embodiments, or the polyamines depicted in Table 1. The covalent bond can be an amide bond, an amine bond, or any other suitable covalent bond. The polyamine analogs can be bound to the porphyrin compound at positions including, but not limited to, any of the peripheral positions of the porphyrin macrocycle such as the β -pyrrole positions or the meso carbons of the macrocycle. Non-limiting examples of these compounds are given in Table 3.

15 In one embodiment, the porphyrin-polyamine compounds are of the formula



where at least one of J₁-J₈ is independently selected from the group consisting of -K₁-G-L-(N(P)-A)_n-K₂; where K₁ is independently selected from the group consisting of C₁-C₈ alkyl and where the valence to the left of K₁ attaches to the porphyrin ring; where G is -O-, -(C=O)-, -C(=O)-O-, -O-(C=O)-, -O-(C=O)-O-, -O-(C=O)-N-, -N-(C=O)-O-, or a

nonentity; where L is C₁-C₈ alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloaryl, C₁-C₈ alkoxy, C₁-C₈ alkyl-C₃-C₈ cycloalkyl, C₁-C₈ alkyl-C₃-C₈ cycloaryl, C₁-C₈ alkoxy-C₃-C₈ cycloaryl, C₃-C₈ cycloalkyl-C₃-C₈ cycloaryl, C₃-C₈ cycloalkyl-C₁-C₈ alkyl, C₃-C₈ cycloaryl-C₁-C₈ alkyl, C₃-C₈ cycloaryl-C₁-C₈ alkoxy, C₃-C₈ cycloaryl-C₃-C₈ cycloalkyl, or a nonentity; each A is independently selected from the group consisting of C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloaryl, C₃-C₈ cycloalkenyl, and C₃-C₈ cycloalkynyl; P is selected from the group consisting of H and C₁-C₈ alkyl; n is an integer from 2 to 8; and K₂ is independently selected from the group consisting of H, C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloaryl, C₃-C₈ cycloalkenyl, C₃-C₈ cycloalkynyl, C₁-C₈ alkanol, C₃-C₈ cycloalkanol, and C₃-C₈ hydroxyaryl; where the remainder of the groups J₁-J₈ are each independently selected from the group consisting of H, K₃, or K₄-COOH, where K₃ is independently selected from the group consisting of C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloaryl, C₃-C₈ cycloalkenyl, C₃-C₈ cycloalkynyl, C₁-C₈ alkanol, C₃-C₈ cycloalkanol, and C₃-C₈ hydroxyaryl; and K₄ is independently selected from the group consisting of C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloaryl, C₃-C₈ cycloalkenyl, and C₃-C₈ cycloalkynyl; and any salt or stereoisomer thereof.

Additional embodiments include those where K₁ and each A are independently selected from the group consisting of C₁-C₈ alkyl, K₂ is independently selected from the group consisting of H and C₁-C₈ alkyl, and the remainder of the groups J₁-J₈ are each independently selected from the group consisting of H, K₃, or K₄-COOH, where K₃ and K₄ are independently selected from the group consisting of C₁-C₈ alkyl. In additional embodiments, K₁ is -CH₂-CH₂- or -CH₂-CH₂-CH₂- . In additional embodiments, n is 4. In yet additional embodiments, J₃, J₄, J₇ and J₈ of the formula depicted above are independently selected from the group consisting of C₁-C₃ alkyl; J₅ and J₆ are independently selected from the group consisting of C₁-C₃ alkyl and C₁-C₃ alkyl-COOH; and J₁ and J₂ are independently selected from the group consisting of C₁-C₃ alkyl-G-N(P₁)-A-(NH-A)_{n1}-K₅; where G is -(C=O)- or a nonentity; P₁ is H or C₁-C₃ alkyl; each A is independently selected from the group consisting of C₁-C₈ alkyl; n₁ is 3 or 4; and K₅ is independently selected from the group consisting of H and C₁-C₈ alkyl.

The invention also includes methods of treating an indication in an individual comprising the step of administering to the individual a therapeutic amount of a porphyrin-polyamine conjugate of the invention.

5 The invention also provides methods of treating an indication comprising the step of administering to the individual an effective amount of a composition comprising a polyamine analog, preferably a conformationally restricted polyamine analog.

In one method, the indication is cancer. In various embodiments, the cancer affects cells of the bladder, blood, brain, breast, colon, digestive tract, lung, ovaries, pancreas, prostate gland, or skin. In other embodiments, the indication can also include, but is not limited to, Alzheimer's disease, epilepsy, multiple sclerosis, problems associated with tissue grafts and organ transplants, psoriasis, restenosis, stomach ulcers, or tissue overgrowth after surgery. In other embodiments, the indication is an infection or infestation of parasites, bacteria, fungi or insects. The polyamine analog or porphyrin-polyamine conjugate is selected from the foregoing groups of compounds.

15

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph depicting the *in vitro* effect of increasing concentrations of BE-4444 (○), SL-11121 (●), SL-11122 (□), SL-11123 (■), SL-11126 (Δ), SL-11127 (▲), SL-20 11128 (◊), SL-11129 (◆), SL-11130 (▽), SL-11133 (▼) on the survival of cultured human prostate cancer cells PC3.

ED₅₀ of BE-4444 = 0.6 μM, SL-11121 = 0.52 μM, SL-11122 >31.25 μM,

SL-11123 >31.25 μM, SL-11126 = 0.2 μM SL-11127 >31.25 μM,

SL-11128 = 0.5 μM, SL-11129 = 1.7 μM, SL-11130 >31.25 μM, and

25 SL-11133 >31.25 μM.

Fig. 2 is a graph depicting the *in vitro* effect of increasing concentrations of BE-4444 (○), SL-11121 (●), SL-11122 (□), SL-11123 (■), SL-11126 (Δ), SL-11127 (▲), SL-11128 (◊), SL-11129 (◆), SL-11130 (▽), and SL-11133 (▼) on the survival of cultured human prostate cancer cells DU145.

30 ED₅₀ of BE-4444 = 0.07 μM, SL-11121 = 0.08 μM, SL-11122 = 0.08 μM,

SL-11123 = 0.51 μM, SL-11126 = 0.51 μM SL-11127 0.22 μM,

SL-11128 = 0.14 μ M, SL-11129 = 0.32 μ M, SL-11130 = 0.43 μ M, and
SL-11133 = 0.34 μ M.

Fig. 3 is a graph depicting the *in vitro* effect of increasing concentrations of BE-4444 (O), SL-11121 (●), SL-11122 (□), SL-11123 (■), SL-11126 (Δ), SL-11127 (\blacktriangle), SL-11128 (\diamond), SL-11129 (\blacklozenge), SL-11130 (\triangledown), and SL-11133 (\blacktriangledown) on the survival of cultured human prostate cancer cells DUPRO.

ED₅₀ of BE-4444 = 0.2 μ M, SL-11121 = 0.4 μ M, SL-11122 = 0.56 μ M,

SL-11123 >31.25 μ M, SL-11126 = 1.1 μ M, SL-11127 1.3 μ M,

SL-11128 = 1.28 μ M, SL-11129 >31.25 μ M, SL-11130 >31.25 μ M, and

SL-11133 = 31.25 μ M.

Fig. 4 is a graph depicting the *in vitro* effect of increasing concentrations of BE-4444 (O), SL-11121 (●), SL-11126 (Δ), SL-11128 (\diamond), on the survival of cultured human prostate cancer cells LNCAP.

ED₅₀ of BE-4444 = 0.14 μ M, SL-11121 = 0.14 μ M, SL-11126 = 0.55 μ M and

SL-11128 = 0.3 μ M.

Fig. 5 is a graph depicting the *in vitro* effect of increasing concentrations of BE-4444 (O), SL-11121 (●), SL-11122 (□), SL-11123 (■), SL-11126 (Δ), SL-11127 (\blacktriangle), and SL-11128 (\diamond) on the survival of cultured human colon cancer cells HT29.

ED₅₀ of BE-4444 = 0.5 μ M, SL-11121 = 0.8 μ M, SL-11122 = 0.8 μ M,

SL-11123 = 10.42 μ M, SL-11126 = 1.5 μ M, SL-11127 = 2.91 μ M, and

SL-11128 = 1.35 μ M.

Fig. 6 is a graph depicting the *in vitro* effect of increasing concentrations of BE-4444 (O), SL-11121 (●), SL-11122 (□), SL-11123 (■), and SL-11126 (Δ) on the survival of cultured human lung cancer cells A549.

ED₅₀ of BE-4444 >31.25 μ M, SL-11121 >31.25 μ M, SL-11122 >31.25 μ M,

SL-11123 >31.25 μ M, and SL-11126 >31.25 μ M.

Fig. 7 is a graph depicting the *in vitro* effect of increasing concentrations of BE-4444 (O), SL-11121 (●), SL-11122 (□), SL-11123 (■), and SL-11126 (Δ) on the survival of cultured human breast cancer cells MCF7.

ED₅₀ of BE-4444 >31.25 μ M, SL-11121 = 17.0 μ M, SL-11122 >31.25 μ M,

SL-11123 >31.25 μ M, and SL-11126 = 0.7 μ M.

Fig. 8 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11105 (●), SL-11124 (○), SL-11132 (■), and BE-333 (□) on the survival of cultured human prostate cancer cells PC3.

ED₅₀ of SL-11105 >31.25 μM, SL-11124 >31.25 μM, SL-11132 >31.25 μM

5 and BE-333 = 0.34 μM.

Fig. 9 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11105 (●), SL-11124 (○), SL-11132 (■), and BE-333 (□) on the survival of cultured human prostate cancer cells DU145.

ED₅₀ of SL-11105 = 1.6 μM, SL-11124 >31.25 μM, SL-11132 = 0.015 μM

10 and BE-333 = 0.12 μM.

Fig. 10 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11105 (●), SL-11124 (○), SL-11132 (■), and BE-333 (□) on the survival of cultured human prostate cancer cells DUPRO.

ED₅₀ of SL-11105 = 0.43 μM, SL-11124 >31.25 μM, SL-11132 >31.25 μM

15 and BE-333 = 0.9 μM.

Fig. 11 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11105 (●), SL-11124 (○), and BE-333 (□) on the survival of cultured human colon cancer cells HT29.

ED₅₀ of SL-11105 = 25.2 μM, SL-11124 >31.25 μM, and BE-333 = 0.3 μM.

20 Fig. 12 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11105 (●), SL-11124 (○), and BE-333 (□) on the survival of cultured human lung cancer cells A549.

ED₅₀ of SL-11105 = 0.43 μM, SL-11124 >31.25 μM, and BE-333 = 0.3 μM.

Fig. 13 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11105 (●), SL-11124 (○), and BE-333 (□) on the survival of cultured human breast cancer cells MCF7.

ED₅₀ of SL-11105 >31.25 μM, SL-11124 >31.25 μM, and BE-333 = 3.7 μM.

Fig. 14 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11105 (●) and BE-333 (□) on the survival of cultured human brain tumor cells U251

30 MG NCI.

ED₅₀ of SL-11105 = 25.9 μM, and BE-333 = 0.23 μM.

Fig. 15A is a graph depicting the *in vitro* effect of increasing concentrations of SL-11093 (●), SL-11098 (○), SL-11099 (■), SL-11100 (□), SL-11101 (▲), SL-11102 (Δ), and BE-444 (◆) on the survival of cultured human prostate cancer cells PC3.

ED₅₀ of SL-11093 = 1.6 μM, SL-11098 = 1.4 μM, SL-11099 = 2.5 μM,

5 SL-11100 = 4.7 μM, SL-11101 = 7.7 μM, SL-11102 >31.25 μM and

BE-444 = 0.7 μM.

Fig. 15B is a graph depicting the *in vitro* effect of increasing concentrations of SL-11103 (●), SL-11104 (○), SL-11108 (■), SL-11114 (□), SL-11118 (▲), SL-11119 (Δ), and BE-444 (◆) on the survival of cultured human prostate cancer cells PC3.

10 ED₅₀ of SL-11103 >31.25 μM, SL-11104 >31.25 μM, SL-11108 = 2.2 μM,

SL-11114 = 0.7 μM, SL-11118 = 1.65 μM, SL-11119 >31.25 μM and

BE-444 = 0.7 μM

Fig. 16A is a graph depicting the *in vitro* effect of increasing concentrations of SL-11093 (●), SL-11098 (○), SL-11099 (■), SL-11100 (□), SL-11101 (▲), SL-11102 (Δ), and BE-444 (◆) on the survival of cultured human prostate cancer cells DU145.

ED₅₀ of SL-11093 = 0.016 μM, SL-11098 = 0.02 μM, SL-11099 = 0.014 μM,

SL-11100 = 0.021 μM, SL-11101 = 0.22 μM, SL-11102 = 0.03 μM and

BE-444 = 0.03 μM.

Fig. 16B is a graph depicting the *in vitro* effect of increasing concentrations of SL-11103 (●), SL-11104 (○), SL-11108 (■), SL-11114 (□), SL-11118 (▲), SL-11119 (Δ), and BE-444 (◆) on the survival of cultured human prostate cancer cells DU145.

ED₅₀ of SL-11103 = 2.8 μM, SL-11104 = 9.4 μM, SL-11108 = 0.13 μM,

SL-11114 = 0.13 μM, SL-11118 = 0.05 μM, SL-11119 0.08 μM and

BE-444 = 0.03 μM.

25 Fig. 17A is a graph depicting the *in vitro* effect of increasing concentrations of SL-11099 (■), SL-11100 (□), SL-11101 (▲), SL-11102 (Δ), and BE-444 (◆) on the survival of cultured human prostate cancer cells DUPRO.

ED₅₀ of = SL-11099 = 0.08 μM, SL-11100 = 0.3 μM, SL-11101 = 0.85 μM,

SL-11102 = 0.15 μM and BE-444 = 0.2 μM.

Fig. 17B is a graph depicting the *in vitro* effect of increasing concentrations of SL-11108 (■), SL-11114 (□), SL-11118 (▲), SL-11119 (△), and BE-444 (◆) on the survival of cultured human prostate cancer cells DUPRO.

ED₅₀ of SL-11108 = 0.98 μM, SL-11114 = 0.64 μM, SL-11118 = 0.25 μM,

5 SL-11119 = 0.44 μM and BE-444 = 0.2 μM.

Fig. 18A is a graph depicting the *in vitro* effect of increasing concentrations of SL-11093 (●), SL-11098 (○), SL-11099 (■), SL-11100 (□), and BE-444 (◆) on the survival of cultured human prostate cancer cells LNCAP.

ED₅₀ of SL-11093 = 0.21 μM, SL-11098 = 0.17 μM, SL-11099 = 0.21 μM,

10 SL-11100 = 0.7 μM, and BE-444 = 0.1 μM.

Fig. 18B is a graph depicting the *in vitro* effect of increasing concentrations of SL-11108 (■), SL-11114 (□), SL-11118 (▲), and BE-444 (◆) on the survival of cultured human prostate cancer cells LNCAP.

ED₅₀ of SL-11108 = 7.7 μM, SL-11114 = 3.0 μM, SL-11118 = 0.21 μM,

15 and BE-444 = 0.1 μM.

Fig. 19A is a graph depicting the *in vitro* effect of increasing concentrations of SL-11093 (●), SL-11098 (○), SL-11099 (■), SL-11100 (□), SL-11101 (▲), SL-11102 (△), and BE-444 (◆) on the survival of cultured human colon cancer cells HT29.

ED₅₀ of SL-11093 = 0.4 μM, SL-11098 = 0.4 μM, SL-11099 = 1.0 μM,

20 SL-11100 = 2.0 μM, SL-11101 = 5.2 μM. SL-11102 = 0.73 μM and

BE-444 = 0.93 μM.

Fig. 19B is a graph depicting the *in vitro* effect of increasing concentrations of SL-11103 (●), SL-11104 (○), SL-11108 (■), SL-11114 (□), SL-11118 (▲), SL-11119 (△), and BE-444 (◆) on the survival of cultured human colon cancer cells HT29.

25 ED₅₀ of SL-11103 = 29.4 μM, SL-11104 = 25.8 μM, SL-11108 = 2.0 μM,

SL-11114 = 3.6 μM, SL-11118 = 0.98 μM, SL-11119 = 0.97 μM and

BE-444 = 0.93 μM.

Fig. 20A is a graph depicting the *in vitro* effect of increasing concentrations of SL-11093 (●), SL-11098 (○), SL-11099 (■), SL-11100 (□), SL-11101 (▲), SL-11102 (△), and BE-444 (◆) on the survival of cultured human lung cancer cells A549.

ED₅₀ of SL-11093 = 0.26 μM, SL-11098 = 0.29 μM, SL-11099 = 0.51 μM,

SL-11100 = 0.65 μM , SL-11101 = 2.2 μM , SL-11102 = 0.15 μM and BE-444 = 0.15 μM .

Fig. 20B is a graph depicting the *in vitro* effect of increasing concentrations of SL-11103 (●), SL-11104 (○), SL-11108 (■), SL-11114 (□), SL-11118 (▲), and BE-444 (◆) on the survival of cultured human lung cancer cells A549.

ED₅₀ of SL-11103 = 12.4 μM , SL-11104 > 31.25 μM , SL-11108 > 31.25 μM , SL-11114 > 31.25 μM , SL-11118 = 0.214 μM and BE-444 = 0.15 μM .

Fig. 21A is a graph depicting the *in vitro* effect of increasing concentrations of SL-11093 (●), SL-11098 (○), SL-11099 (■), SL-11100 (□), SL-11101 (▲), SL-11102 (△), and BE-444 (◆) on the survival of cultured human breast cancer cells MCF7.

ED₅₀ of SL-11093 = 0.66 μM , SL-11098 > 31.25 μM , SL-11099 = 26.3 μM , SL-11100 > 31.25 μM , SL-11101 > 31.25 μM SL-11102 > 31.25 μM and BE-444 > 31.25 μM .

Fig. 21B is a graph depicting the *in vitro* effect of increasing concentrations of SL-11103 (●), SL-11104 (○), SL-11108 (■), and BE-444 (◆) on the survival of cultured human breast cancer cells MCF7.

ED₅₀ of SL-11103 > 31.25 μM , SL-11104 > 31.25 μM , SL-11108 > 31.25 μM , and BE-444 > 31.25 μM .

Fig. 22A is a graph depicting the *in vitro* effect of increasing concentrations of SL-11093 (●), SL-11098 (○), SL-11099 (■), SL-11100 (□), SL-11101 (▲), SL-11102 (△), and BE-444 (◆) on the survival of cultured human brain tumor cells U251 MG NCI.

ED₅₀ of SL-11093 = 0.07 μM , SL-11098 = 0.1 μM , SL-11099 = 0.11 μM , SL-11100 = 0.22 μM , SL-11101 = 1.7 μM , SL-11102 = 0.15 μM and BE-444 = 0.2 μM .

Fig. 22B is a graph depicting the *in vitro* effect of increasing concentrations of SL-11103 (●), SL-11104 (○), SL-11108 (■), and BE-444 (◆) on the survival of cultured human brain tumor cells U251 MG NCI.

ED₅₀ of SL-11103 = 9.5 μM , SL-11104 = 14.71 μM , SL-11108 = 2.0 μM , and BE-444 = 0.2 μM .

Fig. 23 is a graph depicting the *in vitro* effect of increasing concentrations of

SL-11091 (●), SL-11094 (○), and BE-343 (■) on the survival of cultured human prostate cancer cells PC3.

ED₅₀ of SL-11091 >31.25 μM, SL-11094 >31.25 μM, and BE-343 = 0.24 μM.

5 Fig. 24 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11091 (●), SL-11094 (○), and BE-343 (■) on the survival of cultured human prostate cancer cells DU145.

ED₅₀ of SL-11091 = 4.33 μM, SL-11094 = 15.4 μM, and BE-343 = 0.044 μM.

10 Fig. 25 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11091 (●), SL-11094 (○), and BE-343 (■) on the survival of cultured human colon cancer cells HT29.

ED₅₀ of SL-11091 >31.25 μM, SL-11094 = 28.8 μM, and BE-343 = 0.6 μM.

15 Fig. 26 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11091 (●), SL-11094 (○), and BE-343 (■) on the survival of cultured human lung cancer cells A549.

ED₅₀ of SL-11091 >31.25 μM, SL-11094 >31.25 μM, and BE-343 = 0.2 μM.

20 Fig. 27 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11091 (●), SL-11094 (○), and BE-343 (◆) on the survival of cultured human breast cancer cells MCF7.

ED₅₀ of SL-11091 >31.25 μM, SL-11094 >31.25 μM, and BE-343 = 0.5 μM.

25 Fig. 28 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11091 (●), SL-11094 (○), and BE-343 (■) on the survival of cultured human brain tumor cells U251 MG NCI.

ED₅₀ of SL-11091 >31.25 μM, SL-11094 >31.25 μM, and BE-343 = 0.14 μM.

30 Fig. 29 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11141(●), SL-11144 (□), SL-11150 (■) on the survival of cultured human prostate cancer cells PC3.

ED₅₀ of SL-11141>31.25 μM, SL-11144 = 0.3 μM, and SL-11150 = 0.5 μM.

Fig. 30 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11141 (●), SL-11144 (□), SL-11150 (■) on the survival of cultured human prostate cancer cells DU145.

ED₅₀ of SL-11141 = 0.13 μM, SL-11144 = 0.1 μM, and SL-11150 = 0.11 μM.

Fig. 31 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11141 (●), SL-11144 (□), SL-11150 (■) on the survival of cultured human prostate cancer cells DUPRO.

ED₅₀ of SL-11141 = 0.71 μM, SL-11144 = 0.36 μM, and SL-11150 = 0.48 μM.

5 **Fig. 32** is a graph depicting the *in vitro* effect of increasing concentrations of SL-11141 (●), SL-11144 (□), SL-11150 (■) on the survival of cultured human prostate cancer cells LNCAP.

ED₅₀ of SL-11141 = 0.07 μM, SL-11144 = 0.20 μM, and SL-11150 = 0.23 μM.

10 **Fig. 33** illustrates synthetic methodology used to prepare the compounds of the invention.

Fig. 34 illustrates additional synthetic methodology used to prepare the compounds of the invention.

15 **Fig. 35** illustrates additional synthetic methodology used to prepare the compounds of the invention.

Fig. 36 illustrates additional synthetic methodology used to prepare the compounds of the invention.

Fig. 37 illustrates additional synthetic methodology used to prepare the compounds of the invention.

20 **Fig. 38** illustrates additional synthetic methodology used to prepare the compounds of the invention.

Fig. 39 illustrates additional synthetic methodology used to prepare the compounds of the invention.

25 **Fig. 40A** illustrates additional synthetic methodology used to prepare the compounds of the invention.

Fig. 40B illustrates additional synthetic methodology used to prepare the compounds of the invention.

Fig. 41 illustrates synthetic methodology used to prepare porphyrin-polyamine conjugates of the invention.

30 **Fig. 42** illustrates additional synthetic methodology used to prepare porphyrin-polyamine conjugates of the invention.

Fig. 43 illustrates additional synthetic methodology used to prepare porphyrin-polyamine conjugates of the invention.

Fig. 44 illustrates additional synthetic methodology used to prepare porphyrin-polyamine conjugates of the invention.

Fig. 45 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11162 on the survival of human prostate cancer cells DuPro. The concentrations of SL-11162 are plotted in the X-axis and the corresponding surviving fractions of cells are plotted in the Y-axis.

Fig. 46 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11184 on the survival of human prostate cancer cells DuPro. The concentrations of SL-11184 are plotted in the X-axis and the corresponding surviving fractions of cells are plotted in the Y-axis.

Fig. 47 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11202 on the survival of human prostate cancer cells DuPro. The concentrations of SL-11202 are plotted in the X-axis and the corresponding surviving fractions of cells are plotted in the Y-axis.

Fig. 48 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11184 on the survival of human prostate cancer cells PC-3. The concentrations of SL-11184 are plotted in the X-axis and the corresponding surviving fractions of cells are plotted in the Y-axis.

Fig. 49 is a graph depicting the *in vitro* effect of increasing concentrations of SL-11202 on the survival of human prostate cancer cells PC-3. The concentrations of SL-11202 are plotted in the X-axis and the corresponding surviving fractions of cells are plotted in the Y-axis.

25

MODES FOR CARRYING OUT THE INVENTION

The present invention encompasses novel conformationally restricted polyamine analogs and compositions comprising these compounds. These analogs are useful as anti-proliferatives for suppressing cell proliferation. The analogs find use in treatment of various diseases, including use as anti-cancer agents in the treatment of various cancers.

30

The analogs are also useful as anti-microbial agents. Novel polyamine analogs include the compounds shown in the synthetic schemes (Figures 33-40) and Table 1.

The invention also includes polyamine-porphryin conjugates. These conjugates include the compounds depicted in Table 2 and those shown in the synthetic schemes (Figures 41-44).

5 *Definitions*

By "polyamine analog" is meant an organic cation structurally similar but non-identical to polyamines such as spermine and/or spermidine and their precursor, diamine putrescine. By a "polyamine", a term well-understood in the art, is meant any of a group of aliphatic, straight-chain amines derived biosynthetically from amino acids; polyamines are reviewed in Marton et al. (1995) *Ann. Rev. Pharm. Toxicol.* 35:55-91. Polyamine analogs can be branched or un-branched. Polyamine analogs include, but are not limited to, BE-4444 [1,19-bis (ethylamino)-5,10,15-triazaanonadecane]; BE-333 [N1,N11-diethylnorspermine; DENSPM; 1,11-bis (ethylamino)-4,8-diazaundecane; thermine; Warner-Parke-Davis]; BE-33 [N1,N7-bis(ethyl) norspermidine]; BE-34 [N1,N8-bis(ethyl) spermidine]; BE-44 [N1,N9-bis(ethyl) homospermidine]; BE-343 [N1,N12-bis(ethyl) spermine; diethylspermine-N1-N12; DESPM]; BE-373 [N,N'-bis (3-ethylamino) propyl]-1,7-heptane diamine, Merrell-Dow]; BE-444 [N1,N14-bis(ethyl) homospermine; diethylhomospermine-N1-N14]; BE-3443 [1,17-bis(ethylamino)-4,9,14-triazaheptadecane]; and BE-4334 [1,17-bis(ethylamino)-5,9,13-triazaheptadecane]; 1,12-Me₂-SPM [1,12-dimethylspermine]. Additional polyamine analogs are disclosed in International Patent Application WO 98/17624 and U.S. Patent No. 5,889,061. Various novel polyamine analogs are illustrated in the synthetic schemes in Figs. 33-40, and Table 1.

By "conformationally restricted" is meant that, in a polyamine analog, at least two amino groups in the molecule are locked or limited in spatial configuration relative to each other. The amino groups within the molecule may be primary, secondary, tertiary, or quartenary, and are preferably primary or secondary amino groups. The relative movement of two amino groups can be restricted, for example, by incorporation of a cyclic or unsaturated moiety between them (exemplified, but not limited to, a ring, such as a three-carbon ring, four-carbon ring, five-carbon-ring, six-carbon ring, or a double or triple bond, such as a double or triple carbon bond). Groups restricting conformational flexibility by means of steric hindrance, yet structurally favorable to the anti-proliferative, anti-cancer, or anti-microbial effects, can also be used according to the invention. A "conformationally

restricted" polyamine analog can comprise at least two amino groups which are conformationally restricted relative to each other, but can also further comprise amino groups which are not conformationally restricted relative to each other. Flexible molecules such as spermine and BE-444 can have a myriad of conformations and are therefore not 5 conformationally restricted. Conformationally restricted polyamine analogs include, but are not limited to, the compounds listed in Table 1 and illustrated in Figures 33-40.

An "indication" includes any symptom or the like which points out a suitable remedy or treatment or which shows the presence of a disease or other unhealthful condition. As used herein, an "indication" also includes a "disease" itself, where a disease 10 is a condition of an organ, part, structure or system of the body in which there is incorrect function resulting from the effect(s) of heredity, infection, diet and/or environment, and/or other causes. The indication can include cancer. By "cancer" is meant the abnormal presence of cells which exhibit relatively autonomous growth, so that they exhibit an aberrant growth phenotype characterized by a significant loss of cell proliferation control.

15 Cancerous cells can be benign or malignant. In various embodiments, the cancer affects cells of the bladder, blood, brain, breast, colon, digestive tract, lung, ovaries, pancreas, prostate gland, or skin. In other embodiments, the indication can also include, but is not limited to, Alzheimer's disease, epilepsy, multiple sclerosis, problems associated with tissue grafts and organ transplants, psoriasis, restenosis, stomach ulcers, or tissue 20 overgrowth after surgery. In other embodiments, the indication is an infection or infestation of parasites, bacteria, fungi or insects.

An "individual" is a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, farm animals, sport animals, rodents, primates, and pets. Preferably, the individual is known or suspected to be afflicted by cancer or 25 another disease treatable with a polyamine analog.

An "effective amount" or "therapeutic amount" is an amount sufficient to effect beneficial or desired clinical results. An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount of a polyamine analog is an amount that is sufficient to palliate, ameliorate, stabilize, reverse, slow or delay 30 the progression of the disease state. A therapeutic amount of a polyamine of the present invention is an amount sufficient to inhibit proliferation of diseased cells. A polyamine analog is considered to be an effective anti-tumor or anti-cancer agent if it is effective

against at least one type of cancer cell line, even if it is not effective against a different cell line.

As used herein, "treatment" is an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, 5 but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilization (i.e., not worsening) of state of disease, prevention of spread (i.e., metastasis) of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, improvement in quality of enjoyment of life, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as 10 compared to expected survival if not receiving treatment.

"Palliating" a disease means that the extent and/or undesirable clinical manifestations of a disease state are lessened and/or time course of the progression is slowed or lengthened, as compared to not administering polyamine analogs of the present invention. Preferred polyamine analogs for methods of treating and palliating a disease 15 include the compounds illustrated in Table 1.

Polyamine analogs of the present invention

Novel polyamine analogs can be first designed based on current knowledge of polyamines and their analogs. Without wishing to be bound by any particular theory 20 explaining polyamine analog toxicity, the inventors suggest that relevant knowledge includes that related to polyamine interaction with DNA and ability to induce structural changes in nucleic acids. Feuerstein et al. (1991); Gosule et al. (1978) *J. Mol. Biol.* 121:311-326; Behe et al. (1981) *Proc. Natl. Acad. Sci. USA* 78:1619-23; Jain et al. (1989) *Biochem.* 28:2360-2364; and Basu et al. (1990) *Biochem. J.* 269:329-334. Alternatively, a 25 novel polyamine analog can be designed based on its likely ability to inhibit cell growth by suppressing natural polyamine synthesis or depleting the intracellular natural polyamine pool. Porter et al. (1988) in *Advances in Enzyme Regulation*, Pergamon Press, pp. 57-79. Preferably, the novel polyamine analog is conformationally restricted. In the next step, the polyamine analog is tested *in vitro* for efficacy in inhibiting proliferation of diseased cells, 30 such as tumor cells. If the polyamine analog passes this test, it can then be tested in animals, such as nude mice with cancer xenografts. If the compound is found to be efficacious, testing can then proceed to human trials.

The present invention encompasses novel polyamine analogs, such as those shown in Figs. 33-40 and Table 1. Polyamine analogs of the present invention are conformationally restricted. Conformation is a determinant of the spatial arrangement of the pharmacophore or functional groups which interact with receptor binding sites. The 5 latter prefer specific ligand conformations or a specific distribution of conformations. A flexible molecule such as spermine or BE-4444 can have a myriad of conformations. The conformer that binds to the macromolecule (e.g., DNA or RNA) may not necessarily be the one with the lowest energy as determined by spectroscopic methods or theoretically by molecular mechanics calculations. The binding energy of the polyamine binding to the 10 nucleic acid may be overcome with formation of an unstable conformer. Conversely, in the presence of a conformationally rigid analog of a flexible molecule, the host macromolecule might change its overall conformation or the distances from one strand to the other. Hydrogen bonding is the main binding force of either spermine or spermidine associating with the helical region of a tRNA, and very likely also with DNA. Frydman et al. (1992) 15 *Proc. Natl. Acad. Sci. USA* 89:9186-9191; and Fernandez et al. (1994) *Cell Mol. Biol.* 40: 933-944. The secondary amino groups present in the linear spermine analogs BE-343, BE-444, and BE-4444 are the groups most directly involved in the formation of the hydrogen bonds with the paired bases of tRNA. Therefore, these amino groups that usually flank the 20 central four-carbon or three-carbon segment of the polyamine analog can be considered as the pharmacophore of the polyamine analog. When the nitrogens are separated by only a two-carbon segment they are not protonated at pH 7.4 and hence they do not form hydrogen bonds. If these amino groups are locked into various configurations by the incorporation of cyclic or unsaturated moieties into the polyamine analog molecule, a 25 conformationally rigid analog is obtained. When such analogs bind to DNA or tRNA, they will very likely induce a change in the conformation of the nucleic acid strands or loops that may differ from the conformational changes induced by the natural polyamines. A series of conformationally restricted analogs of *bis*-ethyl-spermine that exhibited cytotoxic activities are illustrated in Table 1 and described below.

30 *Porphyrin-polyamine conjugates of the present invention*

The present invention also encompasses novel porphyrin-polyamine conjugates such as those depicted in Figs. 41-44 and in Table 3. These conjugates combine the

selective uptake of porphyrins by tumors with the cytotoxic and cytostatic effects of polyamine analogs. By "porphyrin-polyamine conjugate" is meant any porphyrin compound and any polyamine compound, linked by at least one, and preferably only one, covalent bond. Porphyrin-polyamine conjugates can incorporate any polyamine, whether 5 that polyamine is conformationally restricted or not. Preferably, the polyamine conjugate is conformationally restricted. By "porphyrin" is meant any compound incorporating the porphine ring or derivatives of the porphine ring. Preferred covalent linkages include amide linkages and amine linkages. Examples of porphyrin-polyamine conjugates are depicted in Table 2.

10

Polyamine analogs and inhibition of cell growth

Polyamine analogs of the present invention are likely to be useful for treatment of a variety of diseases, including cancer, Alzheimer's disease, epilepsy, multiple sclerosis, problems associated with tissue grafts and organ transplants, psoriasis, restenosis, stomach 15 ulcers, or tissue overgrowth after surgery, or an infection or infestation of parasites, bacteria, fungi or insects. In order to evaluate the efficacy of a particular novel polyamine for a particular medicinal application, the compounds can be first tested against appropriately chosen test cells *in vitro*. In a non-limiting example, polyamine analogs can be tested against tumor cells, for example, prostate tumor cells. On the basis of the unique 20 nature of polyamine metabolism in the prostate gland, exemplary experiments can utilize cell lines capable of growing in culture as well as *in vivo* in athymic nude mice, such as LNCaP. Horoszewicz et al. (1983) *Cancer Res.* 43:1809-1818. Culturing and treatment of carcinoma cell lines, cell cycle and apoptosis determinations based on flow cytometry; enzyme assays including ODC, SAMDC and SSAT activities; and high pressure liquid 25 chromatography detection and quantitation of natural polyamines and polyamine analogs are described in the art, for example, Mi et al. (1998) *Prostate* 34:51-60; Kramer et al. (1997) *Cancer Res.* 57:5521-27; and Kramer et al. (1995) *J. Biol. Chem.* 270:2124-2132. Evaluations can also be made of the effects of the novel polyamine analog on cell growth and polyamine-related metabolism. Analysis begins with IC₅₀ determinations based on 30 dose-response curves ranging from 0.1 to 1000 µM performed at 72 hr. From these studies, conditions can be defined which produce about 50% growth inhibition and used to: (a) follow time-dependence of growth inhibition for up to 6 days, with particular attention to

decreases in cell number, which may indicate drug-induced cell death; (b) characterize analog effects on cell cycle progression and apoptosis using flow cytometry (analysis to be performed on attached and detached cells); (c) examine analog effects on polyamine metabolic parameters, including the biosynthetic enzymes ODC, SAMDC, the catabolic enzyme SSAT and polyamine pools themselves. Analog effects can be normalized to intracellular concentrations (by HPLC analysis), which also provide an indication of their relative ability to penetrate cells. Marked differences in analog uptake can be further characterized by studying analog ability to utilize and regulate the polyamine transporter, as assessed by competition studies using radiolabeled spermidine, as previously described
5 in Mi et al. (1998).
10

In vivo testing of polyamine analogs and porphyrin-polyamine conjugates

Analogs and conjugates found to have potent or mechanism-based anti-proliferative activity *in vitro* towards cultured carcinoma cells can be evaluated in *in vivo* model
15 systems. The first goal is to determine the relative toxicity of the analogs or conjugates in non-tumor-bearing animals, such as DBA/2 mice. Groups of three animals each can be injected intraperitoneally with increasing concentrations of a compound, beginning at, for example, 10 mg/kg. Toxicity as indicated by morbidity is closely monitored over the first 24 hr. A well-characterized polyamine analog, such as BE-333, can be used as an internal
20 standard in these studies, since a data base has already been established regarding acute toxicity via a single dose treatment relative to chronic toxicity via a daily x 5 d schedule. Thus, in the case of new analogs, single dose toxicity relative to BE-333 is used to project the range of doses to be used on a daily x 5 d schedule.

After the highest tolerated dosage on a daily x 5 d schedule is deduced, antitumor
25 activity is determined. Typically, tumors can be subcutaneously implanted into nude athymic mice by trocar and allowed to reach 100-200 mm³ before initiating treatment by intraperitoneal injection daily x 5 d. Most analogs or conjugates can be given in a range between 10 and 200 mg/kg. Analogs or conjugates can be evaluated at three treatment dosages with 10-15 animals per group (a minimum of three from each can be used for
30 pharmacodynamic studies, described below). Mice can be monitored and weighed twice weekly to determine tumor size and toxicity. Tumor size is determined by multi-directional measurement from which volume in mm³ is calculated. Tumors can be followed until

median tumor volume of each group reaches 1500 mm³ (i.e., 20% of body weight), at which time the animals can be sacrificed. Although the initial anti-tumor studies focuses on a daily x 5 d schedule, constant infusion can be performed via Alzet pump delivery for 5 days since this schedule dramatically improves the anti-tumor activity of BE-333 against
5 A549 human large cell lung carcinoma. Sharma et al. (1997) *Clin. Cancer Res.* 3:1239-1244. In addition to assessing anti-tumor activity, free analog levels or conjugate levels in tumor and normal tissues can be determined in test animals.

Methods of administration of polyamine analogs and porphyrin-polyamine conjugates

10 The polyamine analogs or porphyrin-polyamine conjugates of the present invention can be administered to an individual via any route known in the art, including, but not limited to, those disclosed herein. Preferably administration of the novel polyamine analogs or porphyrin-polyamine conjugates is by intravenous routes. Other methods of administration include but are not limited to, oral, intrarterial, intratumoral, intramuscular,
15 subcutaneous, intraperitoneal, gastrointestinal, and directly to a specific or affected organ. The novel polyamine analogs and porphyrin-polyamine conjugates described herein are administratable in the form of tablets, pills, powder mixtures, capsules, injectables, solutions, suppositories, emulsions, dispersions, food premixes, and in other suitable forms. Additional methods of administration are known in the art. The pharmaceutical dosage
20 form which contains the compounds described herein is conveniently admixed with a non-toxic pharmaceutical organic carrier or a non-toxic pharmaceutical inorganic carrier. Typical pharmaceutically-acceptable carriers include, for example, mannitol, urea, dextrans, lactose, potato and maize starches, magnesium stearate, talc, vegetable oils, polyalkylene glycols, ethyl cellulose, poly(vinylpyrrolidone), calcium carbonate, ethyl
25 oleate, isopropyl myristate, benzyl benzoate, sodium carbonate, gelatin, potassium carbonate, silicic acid, and other conventionally employed acceptable carriers. The pharmaceutical dosage form can also contain non-toxic auxiliary substances such as emulsifying, preserving, or wetting agents, and the like. A suitable carrier is one which does not cause an intolerable side effect, but which allows the novel polyamine analogs or
30 porphyrin-polyamine conjugates to retain its pharmacological activity in the body. Formulations for parenteral and nonparenteral drug delivery are known in the art and are set forth in *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing (1990).

Solid forms, such as tablets, capsules and powders, can be fabricated using conventional tableting and capsule-filling machinery, which is well known in the art. Solid dosage forms can contain any number of additional non-active ingredients known to the art, including excipients, lubricants, dessicants, binders, colorants, disintegrating agents, dry flow
5 modifiers, preservatives, and the like. Liquid forms for ingestion can be formulated using known liquid carriers, including aqueous and non-aqueous carriers, suspensions, oil-in-water and/or water-in-oil emulsions, and the like. Liquid formulations can also contain any number of additional non-active ingredients, including colorants, fragrance, flavorings, viscosity modifiers, preservatives, stabilizers, and the like. For parenteral administration,
10 novel polyamine analogs or porphyrin-polyamine conjugates can be administered as injectable dosages of a solution or suspension of the compound in a physiologically acceptable diluent or sterile liquid carrier such as water or oil, with or without additional surfactants or adjuvants. An illustrative list of carrier oils would include animal and vegetable oils (peanut oil, soy bean oil), petroleum-derived oils (mineral oil), and synthetic
15 oils. In general, for injectable unit doses, water, saline, aqueous dextrose and related sugar solutions, and ethanol and glycol solutions such as propylene glycol or polyethylene glycol are preferred liquid carriers. The pharmaceutical unit dosage chosen is preferably
20 fabricated and administered to provide a final concentration of drug at the point of contact with the cancer cell of from 1 μ M to 10 mM. More preferred is a concentration of from 1 to 100 μ M. As with all pharmaceuticals, the optimal effective concentration of novel polyamine analogs or porphyrin-polyamine conjugates will need to be determined empirically and will depend on the type and severity of the disease, route of administration, disease progression and health and mass or body area of the patient. Such determinations are within the skill of one in the art. Polyamine analogs or porphyrin-polyamine conjugates
25 can be administered as the sole active ingredient, or can be administered together, or in combination with another active ingredient, including, but not limited to, cytotoxic agents, antibiotics, antimetabolites, nitrosourea, vinca alkaloids, polypeptides, antibodies, cytokines, etc.

30 The following examples are provided to illustrate but not limit the invention.

EXAMPLES

Synthesis of conformationally-restricted polyamine analogs and of porphyrin-polyamine conjugates

5 a) Spermine and homospermine analogs containing a conformational restriction.

Scheme 2 exemplifies a N^a, N^ω-bisethyl homospermine analog 7 containing a central *trans*-unsaturated bond. Amide 4 was prepared as described in Scheme 1 by alkylation of amide 1 with bromobutyronitrile to give 2, followed by reduction of the nitrile to the amine 3 that was mesitylsulfonated to 4. Trans-allylic diester 5 was used to alkylate amide 4 and the tetramide 6 was obtained. Deprotection gave the *trans*-tetramide 7 (Scheme 2).

Introduction of a triple bond in the butane segment of homospermine also reduces its mobility. This was achieved by starting with the butyne diester 8 and following the sequence of reactions outlined above (Scheme 3). Schemes 15-20 are further examples of the synthesis of polyamine spermine and homospermine analogs of this type.

15 b) Synthesis of pentamines with conformational restrictions.

Schemes 4-14 are outlines of the syntheses of conformationally restricted pentamines. Scheme 4 depicts the reaction of *cis*-1-chloro-4-phthalimido butene with amide 1 to give 11. Hydrazinolysis of 11 gave 12 which was amidated to 13. Reaction of the latter with 1,4-diiodobutane gave 14, while reaction with equimolar amounts of *cis*-1,4-dichlorobutene gave 15.

20 Amide 4 was alkylated with either 4-chlorobutyronitrile to give 16 or with *cis*-1,4-dichlorobutene to give 19. Nitrile 16 was reduced with hydrogen over Ni Raney to the amine 17 and the latter transformed in to the amide 18 (Scheme 5). Condensation of 18 with the chloroalkyl intermediate 15 gave the pentamide 20 that was deprotected to the pentamine 21 (Scheme 6). Condensation of 18 with the iodoalkyl derivative 14 gave 22 that was deprotected to the pentamine 23 (Scheme 7). Condensation of 18 and 19 gave pentamide 24 that was deprotected to the pentamine 25 (Scheme 8). Using 14 as the alkylating agent, mesylenesulfonamide was dialkylated to give 26, and the latter deprotected to give 27 (Scheme 9). The analogous reaction carried out using 15 as alkylating agent, gave 28 and after deprotection led to the pentamine 29 (Scheme 10).

Alkylation of mesitylenesulfonamide with 19 gave the pentamide 30, which was deprotected to 31 (Scheme 11). When 19 was used to alkylate an equimolar amount of mesitylenesulfonamide then 32 was obtained. Alkylation of 32 with 14 gave 33, that was deprotected to give 34 (Scheme 12). When the chloroalkyl intermediate 15 was used to alkylate one equivalent of mesitylenesulfonamide, then the triamide 35 was obtained. Reaction of 35 with 14 gave 36 which was then deprotected to 37 (Scheme 13). Condensation of 35 and 19 gave the pentamide 38 that was deprotected to 39 (Scheme 14). The above mentioned Schemes describe the synthesis of *cis*-compounds. The same synthetic methodology can be used to obtain the *trans*-isomers, or *cis* and *trans* bonds in different segments within the same molecule.

5 c) **Polyamine analogs with diamidine substituents.**

A new class of polyamine analogs is shown in Scheme 21. They derive from 1,4-dibenzylputrescine, 1,5-dibenzylcadaverine, and 1,6-dibenzylhexanediamine. 15 They are diamidine derivatives, where the diamidine residues are carrier groups that have been shown to be efficient in the transport of drugs into different protozoa. The general procedure of synthesis was based on the condensation of 4-cyanobenzaldehyde with the diaminoalkanes to give the Schiff bases, followed by reduction *in situ* to the corresponding dinitriles 68. The latter were converted to the diamidines 69 through their iminoethers.

20

d) **Synthesis of oligoamines.**

Scheme 22 describes the synthesis of a N-2 hydroxyethyl derivative of a pentamine such as 75. Starting with 18, alkylation with 4-bromobutyronitrile gave 70. Reduction of the nitrile of 70 and mesitylenesulfonylation of the resulting amino group 25 gave 71. It was alkylated again with 4-bromobutyronitrile to give 72, and again reduced and mesitylsulfonylated to give 73. The latter was then alkylated with the benzyl ester of 2-bromoethanol to give 74. Treatment with hydrobromic acid in acetic acid cleaved both the mesitylene sulfonyl protecting groups and the benzyl ether residue to give 75.

Scheme 23 reports the synthesis of a *trans*-decamine 77 and of a *cis*-30 decamine 79. Starting with the pentamide 73 (Scheme 22) and by reaction with *trans*-diester 5 (Scheme 2) the decamide 76 was prepared, which on deprotection gave 77 as a dehydrochloride. In an analogous manner, by condensation of 73 with the *cis*-1,4-

dimesityleneoxy-2-butene, the decamide 78 was prepared, which on deprotection gave 79 as a decahydrochloride.

Scheme 24 outlines the synthesis of a N-2 hydroxyethyl *trans*-decamine 92 and a *cis*-2-hydroxyethyl decamine 95. The procedure repeats almost all the procedures described in the foregoing schemes. The synthesis of 80 proceeded by alkylating BOC-mesitylenesulfonamide with the benzyl ester of 2-bromoethanol. Cleavage of the BOC protecting group leads to 81, alkylation with 4-bromobutyronitrile then gave 82, and after reduction of the nitrile group and reaction with mesitylene sulfonyl chloride the diamide 83 was obtained. Again, alkylation with 4-bromobutyronitrile led to 84, reduction and 10 mesitylsulfonylation gave 85, alkylation of 85 gave 86, reduction and mesitylsulfonylation gave 87, and alkylation, reduction and mesitylsulfonylation performed on 87 gave 89. Alkylation of 73 with *trans*-1,4-dibromo-2-butene gave 90. Alkylation of 89 with 90 gave 91, which after deprotection gave the *trans*- ω -hydroxy-decamine 92. Alkylation of 73 with *cis*-1,4-dichloro-2-butene gave 93. Alkylation of 89 with 93 gave 94. Deprotection of 94 15 gave the *cis*- ω -hydroxy-decamine 95, isomeric with 92.

e) **Synthesis of porphyrin-polyamine conjugates.**

Scheme 25 outlines the synthesis of porphyrin-polyamine. Starting with the above mentioned amide 18 (Scheme 5), alkylation with the benzyl ether of 4-bromobutanol gave 96. Treatment with acid cleaved both the sulfonate and the benzyl protecting groups to give 20 97. Protection of the free amino residues with Boc followed by Mitsunobu reaction using phthalimide gave 99. Cleavage of the phthalyl protecting group with hydrazine hydrate gave polyamine 100. It was condensed with mesoporphyrin IX to give 101, and after cleavage of the Boc protecting group 102 (SL-11162) was obtained.

Scheme 26 outlines the synthesis of a porphyrin-bisethylpolyamine conjugate. The synthesis of the intermediate 105 follows the pattern described above. The primary alcohol is oxidized to the aldehyde 106 in Swern oxidation reaction. The aldehyde 106 was then condensed with ethylamine using a reductive alkylation reaction that gave 107. The latter was coupled with mesoporphyrin IX using amide forming procedures and the resulting 108 30 was deprotected in acid to 109 (SL-11184).

Scheme 27 illustrates on the preparation of a porphyrin-polyamine conjugate, where an amine group tethers the polyamine to the porphyrin. The amide derivative 111 was

prepared as described above and was then reduced using metal hydrides and deprotected to yield 112. The synthesis of the porphyrin-polyamine 112 could also be achieved as shown in the Scheme 28. Reduction of the diester of mesoporphyrin IX under controlled conditions gave the dialdehyde 113. Reductive alkylation of 100 with 113 followed by deprotection also gave 112 (SL-11202).

5

EXAMPLE 1

Synthesis Of Polyamine Compounds

- 10 **Compound 2:** NaH (80%, 1.08 g, 36 mmol) was added to a solution of amide 1 (6.81 g, 30 mmol) in DMF (50 ml) in an ice-water bath under N₂. The mixture was stirred for 1 h and a solution of 4-bromobutyronitrile (4.88 g, 33 mmol) in DMF (10 ml) was added in portions. The mixture was stirred over night at 75°C. The solvent was distilled off, the residue taken up in chloroform washed with a saturated solution of ammonium chloride, dried (Na₂SO₄) and evaporated. The residue was purified by flash chromatography on silica gel (hexane/ethyl acetate 3:1) to yield 8.0 g (90%) of 2 as a colorless oil. ¹H-NMR (CDCl₃) δ 1.05 (t, 3H), 1.90 (m, 2H), 2.30 (b, m, 5H), 2.60 (s, 6H), 3.20 (q, 2H), 3.35 (t, 2H), 6.95 (s, 2H); ¹³C-NMR (CDCl₃): δ 12.50, 20.61, 22.43, 23.60, 31.05, 36.12, 40.39, 43.78, 118.62, 131.79, 132.67, 139.71, 142.41. MS-EI (m/z) 294 (M⁺).
- 15 **Compound 4:** Nitrile 2 (7.8 g, 27 mmol) was dissolved in a mixture of ethanol (150 ml) and concentrated hydrochloric acid (1.5 ml). PtO₂ was added (700 mg) and the mixture was hydrogenated at 50 psi over night. The catalyst was filtered off and the solvent evaporated. The residue (78 g, 98%) was used in the next step without further purification. The free base gave ¹H-NMR (CDCl₃) δ 1.00 (t, 3H), 1.55 (m, 4H), 2.25 (s, 3H), 2.80 (t, 2H), 3.20 (m, 4H), 6.95 (s, 2H); ¹³C-NMR (CDCl₃): δ 12.54, 20.69, 22.53, 24.72, 27.65, 39.92, 40.29, 44.59, 131.71, 133.21, 139.82, 142.09. FAB-MS (m/z) 299 (M⁺ + 1). Mesitylenesulfonyl chloride (8.8 g, 40.5 mmol) in dioxane (30 ml) was added dropwise to a stirred mixture of compound 3 (7.8 g, 27 mmol) dissolved in dioxane (60 ml) and 50% KOH (30 ml) at 0°C. The reaction mixture was allowed to reach 20°C and then kept over night. An excess of water was added and the mixture was extracted with chloroform, dried (Na₂SO₄) and evaporated. The oily residue was crystallized from ethyl acetate/hexane yielding 4; 10.9 g (82%); mp 71.5-72°C. ¹H-NMR (CDCl₃) δ 1.00 (t, 3H), 1.10-1.50 (m,
- 20
- 25
- 30

4H), 2.30 (s, 6H), 2.55, 2.60 (s, 12H), 2.85 (q, 2H), 3.15 (m, 4H), 4.70 (t, 1H), 6.95, 7.00 (s, 4H); ¹³C-NMR (CDCl₃): δ 12.70, 20.92, 21.04, 22.73, 22.92, 24.58, 26.68, 40.04, 42.02, 44.42, 131.91, 133.31, 133.64, 138.99, 140.05, 142.15, 142.35. MS-FAB (m/z) 480 (M⁺).

5 **Compound 5:** (E)-2-Butene-1,4-diyI bis[mesitylenesulfonate] : (E)-2-Butene-1,4-diol (1.76 g, 20 mmol), and benzyltriethylammonium bromide (270 mg, 1 mmol) were dissolved in 30 ml of a 50% potassium hydroxide solution and 30 ml of dioxane. The mixture was stirred at 5°C and mesitylenesulfonyl chloride (8.72 g, 40 mmol) dissolved in 30 ml of dioxane was added dropwise. When the addition was over, stirring was continued
10 for 1 h, water was then added, and the white precipitate was filtered and crystallized from chloroform-hexane to yield 5; 7.0 g (77%); mp 119-120°C. ¹H-NMR (CDCl₃): δ 2.35 (s, 6H), 2.60 (s, 12H), 4.45 (d, 4H), 5.75 (b, 2H), 6.95 (s, 4H); ¹³C-NMR (CDCl₃): δ 20.96, 22.52, 67.96, 127.67, 131.69, 131.74, 139.79, 143.45. MS-EI (m/z), 452 (M⁺), 253, 200, 183. Anal. Calcd for C₂₂H₂₈O₆S₂: C, 58.40; H, 6.19. Found: C, 58.35; H, 6.22.

15 **Compound 6** was synthesized from 5 according to a procedure described elsewhere (Reddy et al., *J. Med. Chem.* 41:4723 (1998)) in 56% yield. ¹H-NMR (CDCl₃): δ 0.95 (t, J=7.15 Hz, 6H, CH₃), 1.34 (m, 8H, CH₂), 2.29 (s, 12H, CH₃), 2.55 (s, 24H, CH₃), 3.09 (m, 12H, NCH₂), 3.72 (d, J=4.53 Hz, 4H, NCH₂), 5.48 (t, J=4.31 Hz, 2H, CH=CH), 6.92 (s, 4H, Ph), 6.93 (s, 4H, Ph); ¹³C-NMR (CDCl₃): δ 12.71, 20.90, 22.71, 22.76, 24.74, 40.04,
20 42.21, 44.56, 45.69, 128.45, 131.88, 132.02, 140.05, 140.16, 142.20, 142.58. MS-FAB (m/z) 1012.8 (M⁺, 100%), 828.7, 646.7, 561, 176.

25 **Compound 7** was obtained from 6 as described elsewhere (Reddy et al., *J. Med. Chem.* 41:4723 (1998)) in 75% yield, mp >230°C. ¹H-NMR (D₂O): δ 1.26 (t, J=12.5 Hz, 6H, 2CH₃), 1.79 (m, 8H, CH₂), 3.12 (m, 12H, NCH₂), 3.80 (d, J=7.16, 4H, NCH₂), 6.10 (m, 2H, CH=CH); ¹³C-NMR (D₂O): δ 12.79, 25.10, 45.19, 48.53, 48.62, 50.36, 130.66. MS-MALDI (m/z): 285.3 (MH⁺, 100%).

30 **Compound 8** was obtained from the commercially available butyne diol. Mesitylenesulfonyl chloride (19.5 g, 90 mmol) in dioxane (30 ml) was added dropwise to a stirred and cooled mixture of butyne diol (2.58 g, 30 mmol), 50% potassium hydroxide (30 ml) and triethylbenzyne ammonium bromide (405 mg, 1.5 mmol). Once the addition was over, the mixture was stirred at room temperature for an additional 3 h. An excess of water was added and the white precipitate was cooled over night, filtered off and dried.

Recrystallization from ethyl acetate/hexane afforded 8.6 g (63%) of 8; mp 105-106°C. ¹H-NMR (CDCl₃): δ 2.30 (s, 6H), 2.60 (s, 12H), 4.50 (s, 4H), 6.95 (s, 4H); ¹³C-NMR (CDCl₃): δ 20.93, 22.48, 56.13, 80.41, 130.65, 131.67, 139.98, 143.67. MS-EI (m/z) 450 (M⁺).

5 Compound 9 was obtained following a procedure analogous to that described for compound 42 (see below). From 450 mg (1 mmol) of diester 8 and 1.05 g (2.2 mmol) of diamide 4, 570 mg (56%) of tetramide 9 was obtained. ¹H-NMR (CDCl₃): δ 0.90 (t, 6H), 1.30 (bs, 8H), 2.20 (s, 12H), 2.45 (s, 24H), 3.05 (m, 12H), 3.75 (s, 4H), 6.87 (s, 8H); ¹³C-NMR (CDCl₃): δ 12.70, 20.78, 22.68, 34.65, 39.97, 44.46, 44.99, 78.62, 131.85, 131.98, 10 132.34, 140.14, 142.13, 142.55. MS-FAB (m/z) 1010 (M[⊕]).

Compound 10 was obtained following a procedure analogous to that described for compound 43 (see below). From 500 mg (0.49 mmol) of tetramide 9, 160 mg (76%) of the tetrahydrochloride 25 was obtained; mp >280°C (decomp). ¹H-NMR (D₂O): δ 1.30 (t, 6H), 1.80 (b, 8H), 2.90-3.25 (m, 12H), 4.05 (s, 4H); ¹³C-NMR (D₂O): δ 13.39, 25.64, 39.26, 15 45.72, 49.00, 49.20, 81.20. MS-MALDI 283 (M⁺ + 1).

Compound 11: Mesylenesulfonylethylamide 1 (3.1 g, 13.65 mmol) was dissolved in anhydrous DMF (30 ml) followed by the addition of NaH (85%, 0.423 g) in several portions. The mixture was stirred at room temperature for 1 h. N-(4-chloro-2-butenyl)-phthalimide (Aldrich, 3.06 g, 13 mmol) in 20 ml of DMF was added to the flask followed by stirring at 80°C over night. The mixture was cooled to room temperature, quenched with H₂O (10 ml), and the solution was evaporated to dryness in vacuo. The solid residue was partitioned between 25 ml H₂O and 25 CHCl₃. The aqueous layer was extracted with CHCl₃ (3 x 25 ml), the organic layers were washed with brine (35 ml), dried (MgSO₄), the solvent was evaporated to afford a gum which solidified upon trituration with hexane to give 11. The ¹H-NMR and ¹³C-NMR spectra showed that 11 was pure enough to be used in the next step without further purification, yield 4.75 g. ¹H-NMR (CDCl₃): δ 1.16 (t, J=7.11 Hz, 3H, CH₃), 2.29 (s, 3H, CH₃), 2.63 (s, 6H, 2CH₃), 3.29 (q, J=7.11 Hz, 2H, CH₂), 4.06 (d, J=5.24 Hz, 2H, NCH₂), 4.26 (d, J=5.72 Hz, 2H, NCH₂), 5.59 (m, 2H, CH=CH), 6.95 (s, 2H, Ph), 7.71 (m, 2H, Ph), 7.83 (m, 2H, Ph); ¹³C-NMR (CDCl₃): δ 13.06, 20.89, 22.72, 34.35, 40.68, 42.01, 123.27, 126.69, 129.47, 131.90, 134.00, 140.24.

Compound 12: Amide 11 (20 g, 46.95 mmol) was dissolved in methanol, hydrazine monohydrate (5 ml, 98.52 mmol) was added and the solution stirred at 55°C for 24 h. Initially it was a homogeneous solution; however, after several hours a white solid precipitated. The mixture was cooled to room temperature, 300 ml of conc. HCl were 5 added slowly (exothermic reaction), and stirring at room temperature was continued for 12 h more. Methanol was evaporated, and the resulting solid was extracted with CHCl₃ (3 x 150 ml). The aqueous layer was neutralized with 50% NaOH, extracted again with CHCl₃ (3 x 100 ml), the combined organic layers were dried (MgSO₄); the solution was evaporated to afford a gum, which solidified under high vacuum to give 12; yield 9.0 g 10 (65%). The compound was purified by column chromatography using hexane, ethyl acetate (7:3) as eluent; mp 167-169°C. ¹H-NMR (CDCl₃): δ 1.0 (t, J=7.1 Hz, 3H, CH₃), 2.28 (s, 3H, CH₃), 2.56 (s, 6H, 2CH₃), 2.62 (br, NH₂), 3.12 (q, J=7.1 Hz, 2H, NCH₂), 3.73 (br, 2H, NCH₂), 3.94 (d, J=6.0 Hz, 2H, NCH₂), 5.80 (m, 2H, CH=CH), 6.92 (s, 2H, Ph); 15 ¹³C-NMR (CDCl₃): δ 12.97, 20.93, 22.74, 36.43, 40.94, 42.08, 124.29, 131.89, 132.00, 132.62, 140.21, 142.67.

Compound 13 was obtained from 12 as described for 4 in 96% yield. It was purified by column chromatography using hexane and ethyl acetate (4:1.5) as eluants; mp 98-99°C; ¹H-NMR (CDCl₃): δ 0.93 (t, J=5.85 Hz, 3H, CH₃), 2.23 (s, 3H, CH₃), 2.24 (s, 3H, CH₃), 2.50 (s, 6H, 2CH₃), 2.56 (s, 6H, 2CH₃), 3.06 (q, J=7.15 Hz, 2H, NCH₂), 3.48 (t, J=5.99 Hz, 2H, 20 NCH₂), 3.68 (d, J=5.72 Hz, 2H, NCH₂), 4.58 (t, J=6.24 Hz, 1H, NH), 5.44 (m, 2H, CH=CH), 6.87 (s, 2H, Ph), 6.89 (s, 2H, Ph); ¹³C-NMR (CDCl₃): δ 12.80, 20.89, 22.64, 22.89, 39.01, 40.59, 41.41, 128.14, 128.46, 131.91, 131.96, 139.08, 140.19, 142.26, 142.54. MS-FAB (m/z) 479.2 (M⁺, 65%), 296.2, 279.1, 267.2, 183.1.

Compound 15: Amide 13 (4.79 g, 10 mmol) was dissolved in anhydrous DMF (40 ml) 25 followed by addition of NaH (0.37 g) in several portions, the mixture stirred at room temperature for 2 h, cis-1,4-dichloro-2-butene (7.5 g, 60 mmol) in 10 ml DMF was added at once, and stirring was continued at 50°C over night. The mixture was cooled to room temperature, quenched with 10 ml H₂O, the solvents were evaporated, and the contents were partitioned between H₂O (50 ml) and CHCl₃ (50 ml). The aqueous layer was 30 extracted with CHCl₃ (3 x 50 ml), the pooled organic layers were dried (MgSO₄), evaporated, and 15 was purified by column chromatography using hexane, ethyl acetate (8.5:1.5) as eluants; yield 5.5 g (97%), mp 106-108°C. ¹H-NMR (CDCl₃): δ 1.03 (t, J=7.33

Hz, 3H, CH₃), 2.30 (s, 6H, 2CH₃), 2.57 (s, 12H, 4CH₃), 3.17 (q, J=7.31 Hz, NCH₂), 3.71 (m, 4H, NCH₂), 3.81 (d, J=6.87 Hz, 2H, NCH₂), 3.95 (d, J=7.70 Hz, 2H, CHCl₂), 5.50 (m, 3H, CH=CH), 5.74 (m, 1H, CH=CH), 6.93 (s, 2H, Ph), 6.95 (s, 2H, Ph); ¹³C-NMR (CDCl₃): δ 12.91, 22.70, 22.74, 38.20, 40.45, 41.60, 42.11, 42.33, 128.17, 128.95, 129.34, 129.40, 131.94, 132.08, 140.23, 140.34, 142.91. MS-FAB (m/z) 566.7 (M⁺, 100%), 153.4, 96.3.

Compound 14 was prepared from 13 and 1,4-diiodobutane as described above for 15. The product was purified by column chromatography using hexanes and ethyl acetate (4:1) as eluant; yield 79%. ¹H-NMR (CDCl₃): δ 1.04 (t, J=7.10 Hz, 3H, CH₃), 1.63 (m, 4H, CH₂), 2.30 (s, 6H, 2CH₃), 2.58 (s, 12H, 4CH₃), 3.04 (t, J=6.50 Hz, 2H, CH₂I), 3.16 (m, 4H, NCH₂), 3.78 (d, J=5.14 Hz, 4H, NCH₂), 5.55 (m, 2H, CH=CH), 6.94 (s, 2H, Ph), 6.95 (s, 2H, Ph); ¹³C-NMR (CDCl₃): δ 5.69, 12.92, 20.95, 22.72, 22.78, 28.25, 30.36, 40.47, 41.59, 42.11, 44.71, 128.34, 129.00, 131.94, 132.06, 132.60, 132.89, 140.15, 140.21, 142.50, 142.71.

Compound 16 was prepared from 4 and 4-bromobutyronitrile as described above for Compound 2 in 94% yield.

¹H NMR(CDCl₃): δ 0.97 (t, J = 7.12Hz, 3H, CH₃), 1.40 (m, 4H, 2CH₂), 1.85 (Pent., m, 2H, CH₂), 2.27 (t, J = 7.17Hz, 2H CH₂CN), 2.30 (s, 6H, 2CH₃), 2.57 (s, 6H, 2CH₃), 2.58 (s, 6H, 2CH₃), 3.13 (m, 6H, NCH₂), 3.28 (t, J = 7.11Hz, 2H, NCH₂), 6.94 (s, 2H, Ph), 6.96 (s, 2H, Ph); ¹³C NMR (CDCl₃): δ 12.55, 14.54, 20.84, 22.64, 22.73, 23.65, 24.43, 24.57, 39.88, 44.31, 44.54, 45.58, 118.69, 131.84, 132.05, 132.73, 133.36, 139.94, 142.20, 142.71.

Compound 17 was prepared from 16 as described above for Compound 3 in 93% yield. ¹H NMR(CDCl₃): δ 1.00 (t, J = 6.92Hz, 3H, CH₃), 1.40 (m, 10H, 4CH₂, NH₂), 2.29 (s, 6H, 2CH₃), 2.57 (b, 14H, 4CH₃, CH₂N), 3.13 (m, 8H, 4CH₂N), 6.93 (s, 4H, 2 Ph); ¹³C NMR (CDCl₃): 12.72, 20.90, 22.72, 22.78, 24.67, 24.80, 30.80, 40.02, 41.61, 44.56, 45.10, 45.38, 131.87, 140.04, 142.21, 142.28; MS-FAB(M/Z) 552.3(M⁺, 100%), 368.2, 299.1, 183.0, 154.0.

Compound 18 was prepared from 17 as described above for Compound 4.

¹H NMR(CDCl₃): δ 0.96 (t, J = 7.13Hz, 3H, CH₃), 1.38 (m, 8H, 4CH₂), 2.29 (s, 9H, 3CH₃), 2.55 (s, 6H, 2CH₃), 2.56 (s, 6H, 2CH₃); 2.59 (s, 6H, 2CH₃), 2.80 (m, 2H, CH₂N), 3.10 (m, 8H, NCH₂), 4.67(t, J = 6.6Hz, 1H, NH), 6.93 (s, 6H, 3 Ph); ¹³C NMR(CDCl₃): δ 12.56, 20.87, 22.70, 22.74, 22.84, 24.40, 26.45, 24.67, 26.62, 39.87, 41.88, 44.45, 45.02,

45.09, 131.86, 131.90, 131.92, 133.12, 133.32, 133.68, 138.91, 139.97, 142.02, 142.21, 142.38; MS-FAB(M/Z): 756.9(M + 23(Na), 100%) 572.8, 390.7, 333.6, 305.6

Compound 19 was prepared from 4 and 1,4-dichloro-2-butene as described above for 15 in 99% yield. ¹H-NMR (CDCl₃): δ 1.01 (t, J=7.11 Hz, 3H, CH₃), 1.38 (m, 4H, CH₂), 2.29

5 (s,3H), 2.30 (s,3H), 2.57 (s, 6H), 2.61 (s, 6H), 3.11 (m, 4H, NCH₂), 3.16 (q, J=7.15 Hz, 2H, NCH₂), 3.81 (d, J=7.17 Hz, 2H, NCH₂), 3.98 (d, J=8.05 Hz, 2H, CH₂Cl), 5.51 (m, 1H, CH=CH), 5.77 (m, 1H, CH-CH), 6.93 (s, 2H, Ph), 6.95 (s, 2H, Ph); ¹³C-NMR (CDCl₃): δ 12.76, 20.91, 22.71, 22.76, 24.74, 38.12, 40.08, 41.85, 44.59, 45.54, 129.14, 129.25, 131.88, 132.02, 140.09, 140.19, 142.21, 142.63. MS-FAB (m/z) 569.3 (M⁺, 20%), 385.2, 10 240.1, 203.3, 183.0, 119 (100%).

Compound 20 was prepared from 18 and 15 following the procedure described above for 15. It was purified by column chromatography using hexanes - ethyl acetate (7:3) as eluant (78% yield). ¹H-NMR (CDCl₃): δ 0.97 (t, J=7.10 Hz, 3H, CH₃), 0.99 (t, J=7.0 Hz, 3H, CH₃), 1.29 (m, 8H, CH₂), 2.29 (s, 15H, CH₃), 2.54, 2.55, 2.59 (s, 30H, CH₃), 3.06 (m, 12H, NCH₂), 3.65 (m, 8H, NCH₂), 5.48 (m, 4H, CH=CH), 6.92 (s, 10H, Ph); ¹³C-NMR (CDCl₃): δ 12.70, 12.83, 20.88, 20.91, 22.65, 22.68, 22.72, 22.74, 24.48, 24.72, 40.04, 40.47, 41.53, 15 42.07, 42.22, 42.34, 44.54, 44.96, 127.94, 128.27, 128.57, 129.20, 131.92, 132.05, 139.96, 140.00, 140.12, 140.16, 140.27, 142.19, 142.25, 142.47, 142.58, 142.87. MS-FAB (m/z) 1263.81 (M⁺, 100%), 1080.01, 898.11, 714.81, 563.

20 Compound 21: Pentamide 20 (0.93 g, 0.735 mmol) was dissolved in 20 ml anhydrous CH₂Cl₂, phenol (3.46 g, 36.77 mmol) was added, followed by HBr in acetic acid (30%, 17.62 ml) and the mixture was stirred over night at 25°C. Water (10 ml) was added to the flask, the aqueous layer was separated, the organic layer was extracted with 5 ml H₂O, and the combined aqueous layers were washed with CH₂Cl₂ (6 x 15 ml). Water was evaporated under vacuum to afford a solid which was dissolved in 1 ml 1N NaOH followed by 1 ml of 50% KOH. This solution was extracted with CHCl₃ (10 x 5 ml). The combined organic layers were dried (MgSO₄), CHCl₃ was evaporated, and the residue dissolved in anhydrous diethyl ether. Anhydrous HCl gas was passed into the solution while cooling at 0°C. A white solid precipitated which was filtered and washed with ether. It was 21 (84%). ¹H-NMR (D₂O): δ 1.29 (t, J=7.32 Hz, 3H, CH₃), 1.31 (t, J=7.24 Hz, 3H, CH₃), 1.79 (m, 8H, CH₂), 3.12 (m, 12H, NCH₂), 3.87 (m, 8H, NCH₂), 5.98 (m, 4H, CH=CH); ¹³C-NMR (D₂O):

δ 13.36, 13.46, 25.66, 25.77, 45.44, 45.74, 46.24, 46.41, 46.84, 49.09, 49.41, 49.70, 129.02, 129.16, 129.47, 129.66. MS-MALDI (m/z) 354.36 (MH⁺, 100%).

Compound 22 was prepared in 51% yield from **18** and **14** as described above for compound **15**. ¹H-NMR (CDCl₃): δ 0.97 (t, J=6.59 Hz, 3H, CH₃), 0.99 (t, J=7.02 Hz, 3H, CH₃), 1.29 (m, 12H, CH₂), 2.29 (s, 15H, CH₃), 2.55 (s), 2.56 (s), 2.57 (s), 3.10 (m, 16H, NCH₂), 3.70 (m, 4H, NCH₂), 5.47 (m, 2H, CH=CH), 6.93 (s, 10H, Ph); ¹³C-NMR (CDCl₃): δ 12.69, 12.83, 20.91, 22.69, 22.71, 22.76, 24.43, 24.70, 40.48, 41.11, 41.48, 44.50, 44.91, 128.13, 128.90, 131.88, 131.94, 132.01, 133.29, 139.95, 140.00, 140.15, 142.22, 142.29, 142.60. MS-FAB (m/z) 1265.91 (M⁺, 100%), 1082.01, 900.11, 716.91, 563.81.

10 **Compound 23** was prepared from **22** in 79% yield as described above for **21**. ¹H-NMR (D₂O): δ 1.29 (t, J=7.29 Hz, 3H, CH₃), 1.30 (t, J=7.30 Hz, 3H, CH₃), 1.78 (m, 12H, CH₂), 3.12 (m, 16H, NCH₂), 3.83 (m, 4H, NCH₂), 5.96 (m, 2H, CH=CH); ¹³C-NMR (D₂O): δ 13.31, 13.42, 25.62, 25.75, 45.38, 45.71, 46.18, 46.76, 49.07, 49.32, 49.69, 129.11, 129.39. MS-MALDI (m/z) 356.38 (MH⁺, 100%).

15 **Compound 24** was prepared from **18** (52% yield) as described. ¹H-NMR (CDCl₃): δ 0.95 (m, 6H, 2CH₃), 1.32 (m, 12H, CH₂), 2.29 (s, 15H, CH₃), 2.55 (s, 30H, CH₃), 3.06 (m, 16H, NCH₂), 3.70 (m, 4H, NCH₂), 5.47 (m, 2H, CH=CH), 6.92 (s, 10H, Ph); ¹³C-NMR (CDCl₃): δ 12.67, 20.90, 22.71, 22.76, 24.43, 24.68, 39.97, 42.08, 44.48, 44.90, 45.61, 128.28, 128.45, 131.87, 131.93, 132.01, 139.96, 140.00, 140.12, 142.21, 142.28, 142.58. MS-FAB (m/z) 1265.91 (M⁺, 100%), 1082.01, 900.11.

Compound 25 was prepared from **24** in 96% yield as described above for **21**. ¹H-NMR (D₂O): δ 1.28 (t, J=7.29 Hz, 6H, 2CH₃), 1.78 (m, 12H, CH₂), 3.09 (m, 16H, NCH₂), 3.84 (m, 4H, NCH₂), 5.96 (m, 2H, CH=CH); ¹³C-NMR (D₂O): δ 13.31, 25.61, 25.73, 45.70, 46.79, 49.05, 49.36, 49.65, 129.19. MS-MALDI (m/z) 356.4 (MH⁺).

25 **Compound 26:** A mixture of KOH (0.25 g), K₂CO₃ (0.25 g) and tetra-n-butyl-ammonium hydrogen bromide (0.05 g) were suspended in 15 ml benzene. Mesitylenesulfonamide (0.199 g, 1 mmol) was added to the suspension and the mixture was heated to 50°C. Iodide **14** (1.98 g, 3 mmol) in 10 ml benzene was added to the flask, the mixture heated under reflux over night, then cooled to room temperature; the inorganic solids were filtered off and washed with benzene (2 x 20 ml). The combined organic layers were washed several times with water until the washings were neutral. The benzene was dried (MgSO₄),

evaporated and the residue purified by column chromatography using hexanes and ethyl acetate (7.5:2.5) as eluant; 25% yield (0.948 g). $^1\text{H-NMR}$ (CDCl_3): δ 1.00 (t, $J=7.18$ Hz, 6H, CH_3), 1.28 (m, 8H, CH_2), 2.29 (s, 15H, CH_3), 2.53 (s), 2.55 (s), 2.57 (s), 3.03 (m, 8H, NCH_2), 3.12 (q, $J=7.13$ Hz, 4H, NCH_2), 3.70 (m, 8H, NCH_2), 5.47 (m, 4H, $\text{CH}=\text{CH}$), 6.93 (s, 10H, Ph); $^{13}\text{C-NMR}$ (CDCl_3): δ 12.78, 20.85, 22.63, 22.69, 24.32, 24.58, 40.41, 41.43, 42.00, 44.76, 45.43, 128.08, 128.83, 131.88, 131.95, 132.77, 132.85, 133.23, 139.90, 140.04, 140.08, 142.22, 142.43, 142.53. MS-FAB (m/z) 1263.81 (M^+ , 100%), 1081, 898.11, 815.01, 561.81, 418.81.

Compound 27 was prepared from 26 in 57% yield as described above for 21. $^1\text{H-NMR}$ (D_2O): δ 1.31 (t, $J=7.31$ Hz, 6H, CH_3), 1.78 (m, 8H, CH_2), 3.15 (m, 12H, NCH_2), 3.83 (m, 8H, NCH_2), 5.96 (m, 4H, $\text{CH}=\text{CH}$); $^{13}\text{C-NMR}$ (CDCl_3): δ 13.43, 25.64, 25.76, 45.39, 46.19, 46.77, 49.35, 49.72, 129.11, 129.41. MS-MALDI (m/z) 354.3 (MH^+ , 100%).

Compound 28 was prepared from 15 and mesitylenesulfonyl amide in 24% yield as described above for 26; mp 57.7°C. $^1\text{H-NMR}$ (CDCl_3): δ 0.99 (t, $J=7.09$ Hz, 6H, CH_3), 2.29 (s, 15H, CH_3), 2.53 (s), 2.55 (s), 3.12 (q, $J=7.09$ Hz, 4H, NCH_2), 3.63 (m, 16H, NCH_2), 5.49 (m, 8H, $\text{CH}=\text{CH}$), 6.93 (s, 10H, Ph); $^{13}\text{C-NMR}$ (CDCl_3): δ 12.85, 20.89, 20.92, 22.66, 40.47, 41.53, 42.19, 128.00, 128.47, 128.58, 129.11, 131.92, 132.05, 140.17, 140.30, 142.46, 142.87. MS-FAB (m/z) 1259.81 (M^+ , 60%), 1075.91, 894.01, 306.51, 153.4 (100%).

Compound 29 was prepared from 28 in 81% yield as described above for 21. $^1\text{H-NMR}$ (D_2O): δ 1.31 (t, $J=7.29$ Hz, 6H, CH_3), 3.15 (q, $J=7.31$ Hz, 4H, NCH_2), 3.84 (m, 4H, NCH_2), 3.90 (m, 12H, NCH_2), 5.98 (m, 8H, $\text{CH}=\text{CH}$); $^{13}\text{C-NMR}$ (D_2O): δ 13.42, 45.41, 46.22, 46.44, 129.07, 129.37, 129.42, 129.58. MS-MALDI (m/z) 350.31 (MH^+).

Compound 30 was prepared from 19 in 25% yield as described above for 26; mp 62.3°C. $^1\text{H-NMR}$ (CDCl_3): δ 0.95 (s, $J=7.17$ Hz, 6H, CH_3), 1.33 (m, 8H, CH_2), 2.29 (s, 15H, CH_3), 2.54 (s), 2.55 (s), 3.07 (m, 12H, NCH_2), 3.65 (m, 8H, NCH_2), 5.48 (m, 4H, $\text{CH}=\text{CH}$), 6.92 (s, 10H, Ph); $^{13}\text{C-NMR}$ (CDCl_3): δ 12.69, 20.90, 22.69, 22.73, 24.70, 40.03, 42.13, 42.30, 44.53, 45.59, 128.11, 128.79, 131.87, 132.00, 140.02, 140.14, 140.28, 142.17, 142.58, 142.85. MS-FAB (m/z) 1263.81 (M^+ , 100%), 1080.01, 898.11, 714.01, 153.

Compound 31 was prepared from 30 in 87% yield as described above for 21. $^1\text{H-NMR}$ (D_2O): δ 1.28 (t, $J=7.32$ Hz, 6H, CH_3), 1.79 (m, 8H, CH_2), 3.10 (m, 12H, NCH_2), 3.87 (m,

8H, NCH₂), 5.98 (m, 4H, CH=CH), ¹³C-NMR (D₂O): δ 12.70, 25.00, 25.13, 45.10, 45.81, 46.21, 48.44, 48.78, 128.44, 128.85. MS-MALDI (m/z) 354.3 (MH⁺).

Compound 32: Mesitylenesulfonamide (1.47 g, 7.38 mmol) was dissolved in 50 ml anhydrous DMF, and NaH (85%, 0.3 g) was added to it under a nitrogen atmosphere. The mixture was stirred at room temperature and 19 (1.40 g, 2.46 mmol) in 25 ml DMF were added. Heating at 65°C continued over night. The mixture was cooled to room temperature, and 10 ml of H₂O were added. The solvents were evaporated and the solid residue was partitioned between 40 ml H₂O and 40 ml CHCl₃. The aqueous layer was extracted with CHCl₃ (2 x 30 ml), the combined organic layers were washed with H₂O (3 x 50 ml), dried (MgSO₄), and evaporated. The residue was purified by column chromatography using hexanes - ethyl acetate (7.5:2.5). 1.7 g (97%) of 32 as a white solid was obtained. ¹H-NMR (CDCl₃): δ 0.94 (t, J=7.10 Hz, 3H, CH₃), 1.30 (m, 4H, CH₂), 2.29 (s), 2.30 (s), 2.55 (s, 12H, CH₃), 2.65 (s, 6H, CH₃), 3.11 (m, 6H, NCH₂), 3.52 (m, 1H, NCH), 3.65 (m, 2H, NCH₂), 3.71 (m, 1H, NCH₂), 4.82 (br, 1H, NH), 5.47 (m, 2H, CH=CH), 6.93 (s, 4H, Ph), 6.96 (s, 2H, Ph); ¹³C-NMR (CDCl₃): δ 12.50, 20.91, 22.71, 22.76, 22.83, 22.91, 24.66, 38.98, 39.85, 42.15, 42.26, 44.50, 128.06, 128.51, 131.86, 131.91, 138.18, 140.00, 140.14, 140.28, 142.17, 142.65.

Compound 33 was prepared from 32 and 14 in 51% yield as described above for 22. ¹H-NMR (CDCl₃): δ 0.99 (s, J=7.19 Hz, 6H, CH₃), 1.33 (m, 8H, CH₂), 2.29 (s, 15H, CH₃), 2.55 (s), 2.57 (s), 3.10 (m, 12H, NCH₂), 3.70 (m, 4H, NCH₂), 3.77 (m, 4H, NCH₂), 5.42 (m, 4H, CH=CH), 6.93 (s, 10H, Ph); ¹³C-NMR (CDCl₃): δ 12.70, 12.71, 20.89, 22.66, 22.72, 22.78, 22.81, 24.60, 26.53, 40.39, 41.37, 41.87, 42.20, 45.47, 128.26, 128.62, 131.78, 131.84, 131.86, 131.92, 132.77, 138.92, 139.96, 140.09, 140.17, 142.57, 142.63.

Compound 34 was prepared from 33 as described above for 21 in 40% yield.

Compound 35 was prepared from 15 in 94% yield as described above for 32.

Compound 36 was prepared from 35 and 14 in 82% yield as described above for 33. ¹H-NMR (CDCl₃): δ 0.99 (t, J=7.11 Hz, 6H, CH₃), 1.33 (m, 4H, CH₂), 2.29 (s, 15H, CH₃), 2.55 (s), 2.57 (s), 3.07 (m, 8H, NCH₂), 3.70 (m, 12H, NCH₂), 5.46 (m, 6H, CH=CH), 6.92 (s, 10H, Ph); ¹³C-NMR (CDCl₃): δ 12.69, 12.80, 20.84, 22.62, 22.68, 22.73, 22.77, 24.58, 26.55, 40.44, 41.51, 41.86, 42.04, 42.24, 45.49, 128.10, 128.25, 128.52, 128.62, 128.82,

131.89, 131.95, 132.79, 138.89, 140.07, 140.14, 140.23, 141.94, 142.44, 142.53, 142.82.

MS-FAB (m/z) 1262.8 (M^+ , 75%), 1080.01, 896, 119 (100%).

Compound 37 was prepared from 36 in 65% yield as described above for 21. 1H -NMR (D₂O): δ 1.31 (t, J=6.97 Hz, 6H, CH₃), 1.79 (m, 4H, CH₂), 3.12 (m, 8H, NCH₂), 3.83 (m, 12H, NCH₂), 5.96 (m, 6H, CH=CH); ^{13}C -NMR (D₂O): δ 13.48, 25.69, 26.76, 41.67, 45.44, 46.24, 46.45, 46.80, 49.41, 129.00, 129.12, 129.45, 129.71. MS-MALDI (m/z) 352.3 (MH $^+$).

Compound 38 was prepared from 35 and 19 in 89% yield as described. 1H -NMR (CDCl₃): δ 0.95 (m, 6H, CH₃), 1.33 (m, 4H, CH₂), 2.29 (s, 15H, CH₃), 2.54 (s), 2.55 (s), 2.57 (s), 3.09 (m, 8H, NCH₂), 3.66 (m, 12H, NCH₂), 5.48 (m, 6H, CH=CH), 6.93 (s, 10H, Ph); ^{13}C -NMR (CDCl₃): δ 12.51, 12.63, 20.84, 20.86, 22.63, 22.65, 22.84, 24.61, 38.92, 40.40, 41.40, 42.11, 42.18, 44.44, 45.48, 127.95, 128.07, 128.49, 128.62, 128.80, 131.76, 131.83, 131.85, 131.88, 132.01, 138.05, 139.01, 140.07, 140.13, 140.24, 142.15, 142.21, 142.87. MS-FAB (m/z) 1263.1 (M^+ , 90%), 1080.1, 896.01, 119 (100%).

Compound 39 was prepared from 38 in 54% yield as described above for 21; mp 270°C (dec.). 1H -NMR (D₂O): δ 1.31 (m, 6H, CH₃), 1.80 (m, 4H, CH₂), 3.10 (m, 8H, NCH₂), 3.86 (m, 12H, NCH₂), 5.98 (m, 6H, CH=CH); ^{13}C -NMR (D₂O): δ 13.30, 13.42, 25.58, 25.70, 45.69, 46.21, 46.43, 46.81, 49.02, 49.37, 129.00, 129.15, 129.37, 129.59. MS-MALDI (m/z): 352.343 (MH $^+$).

Compound 42: NaH (80%, 132 mg, 4.4 mmol) was added to a solution of diamide 41 (1.98 g, 4.4 mmol) in DMF (10 ml). The mixture was stirred at 20°C for 30 minutes and a solution of the diester 40 (Reddy et al. (1998) J. Med Chem., 41:4723) (960 mg, 2 mmol) in DMF (10 ml) was added dropwise. The mixture was stirred at 75°C for 2 h, the solvent was distilled off, the residue was taken in chloroform, washed with a saturated solution of ammonium chloride, dried (Na₂SO₄) and evaporated to dryness. The crude oil was purified by column chromatography using hexane - ethyl acetate (8:2) as running solvent. 1.4 g (70%) was obtained as a glassy oil. ^{13}C -NMR (CDCl₃): δ 20.58, 22.63, 22.80, 32.42, 33.86, 43.16, 45.42, 46.26, 132.75, 133.21, 139.82, 142.40. MS-FAB 984 (M^+),

Compound 43: Phenol (1.86 g, 19.7 mmol) and 30% HBr in glacial acetic acid (35 ml)

were added in that order to a solution of 42 (600 mg, 0.6 mmol) in CH₂Cl₂ (35 ml) at room temperature. The solution was stirred for 24 h, water (30 ml) was added, followed by extraction with methylene chloride (3 x 20 ml). The aqueous layer was evaporated under

reduced pressure and the residue was taken up in 2N NaOH (2 ml) and then 50% KOH (2 ml) followed by extraction with chloroform (6 x 10 ml). After removal of chloroform, the residue was taken up in ethanol (15 ml) and acidified with concentrated hydrochloric acid (0.4 ml). The product 43 (230 mg, 93%) was recrystallized from aqueous ethanol; mp
5 >270°C (decomp). ¹H-NMR (D₂O): δ 1.95 (m, 2H), 2.05-2.25 (m, 6H), 2.75 (s, 6H), 2.90 (b, 2H), 3.10-3.35 (m, 12H); ¹³C-NMR (D₂O): δ 25.21, 25.24, 35.60, 35.64, 47.41, 48.58, 50.87. MS-MALDI (m/z) 240 (M⁺ + 1).

Compound 47: NaH (80%, 132 mg, 4.4 mmol) was added to a solution of diamide 46 (1.98 g, 4.4 mmol) in DMF (10 ml). The mixture was stirred at 20°C for 30 min and a
10 solution of the diester 8 (900 mg, 2 mmol) in DMF (10 ml) was added dropwise. The mixture was stirred at 75°C for 2 h. The solvent was distilled off, the residue was taken up in chloroform, washed with a saturated solution of ammonium chloride, dried (NaSO₄) and evaporated to dryness. The oily residue was crystallized from ethyl acetate/hexane 1.2 g (61%); mp 165-166°C. ¹H-NMR (CDCl₃): δ 1.08 (t, 3H), 1.75 (m 4H), 2.28 (s, 12H), 2.55 (bs, 24H), 3.10 (m, 12H), 3.98 (s, 4H), 6.95 (m, 8H); ¹³C-NMR (CDCl₃): δ 12.70, 20.86,
15 22.64, 25.14, 34.85, 40.22, 42.62, 43.37, 78.80, 131.99, 132.26, 133.21, 140.26, 142.28,
142.71. MS-FAB (m/z) 982 (M⁺).

Compound 48 was obtained as described for 47. From 1.2 g (1.22 mmol) of tetramide 47, 420 mg (86%) of the tetrahydrochloride 48 was obtained; mp >270°C (decomp). ¹H-NMR
20 (D₂O): δ 1.29 (t, 6H), 2.13 (m, 4H), 3.14 (m, 12H), 4.06 (s, 4H); ¹³C-NMR (D₂O): δ
13.34, 25.52, 39.45, 45.90, 45.64, 46.71, 81.32. MS-MALDI (m/z) 255 (M⁺ + 1).

Compound 44 was obtained as described for 47. From 450 mg (1 mmol) of diester 8 and 994 mg (2.2 mmol) of diamide 41, 500 mg (52%) of the tetramide 44 was obtained and crystallized from ethyl acetate - hexane; mp 155-156°C.

Compound 45 was obtained as described for 43. From 500 mg (0.52 mmol) of tetramide 44, 160 mg (82%) of the tetrahydrochloride 45 was obtained; mp >270°C (decomp). ¹H-NMR (D₂O): δ 2.15 (m, 4H), 2.73 (s, 3H), 3.05-3.40 (m, 8H), 4.10 (s, 4H); ¹³C-NMR (D₂O): δ 25.59, 35.66, 45.90, 46.57, 48.61.

Compound 51 is a mixture of *cis/trans*- isomers. ¹H-NMR (D₂O): δ 1.15-2.10 (m, 7H),
30 2.90 (q, 1H), 3.30-3.80 (b, 2H); ¹³C-NMR (D₂O): δ 24.16, 24.97, 28.44, 30.42, 36.58,
37.14, 48.24, 52.27, 55.19, 57.45, 64.55, 67.26.

Compound 52: Mesitylenesulfonylchloride (6.5 g, 30 mmol) in dioxane (10 ml) was added dropwise to a stirred and cooled mixture of amine alcohol 51 (1.15 g, 10 mmol), triethylbenzyl ammonium bromide (135 mg, 0.5 mmol), 50% KOH (10 ml) and dioxane (10 ml). The reaction mixture was left over night at 20 °C with magnetic stirring. An excess of water was added, the solution was extracted with chloroform (3 x 30 ml), dried (Na_2SO_4) and evaporated to dryness. The oily residue was chromatographed on a silica-gel column using hexane:ethyl acetate (8:2) as eluants. Crystallization from ethyl acetate - hexane afforded 1.2 g (25%) of pure 52; mp 167-168°C. $^1\text{H-NMR}$ (CDCl_3): δ 1.35-1.90 (6H), 1.90-2.15 (m, 1H), 2.30, 2.35 (s, 6H), 2.65 (s, 12H), 3.20 (m, 1H), 3.70 (m, 1H), 3.90 (m, 1H), 5.15 (d, 1H), 6.90, 7.00 (s, 4H); $^{13}\text{C-NMR}$ (CDCl_3): δ 20.73, 20.85, 22.15, 22.37, 22.70, 26.94, 32.75, 45.34, 56.09, 70.38, 130.22, 131.57, 133.98, 138.68, 139.64, 142.02, 143.10. MS-EI (m/z) 479 (M^+), 280 ($\text{M}^+ - 199$).

Compound 54: NaH (105 mg, 3.5 mmol) was added to a solution of compound 52 (1.7 g, 3.5 mmol) in DMF (10 ml). The mixture was stirred at 20°C for 30 min and a solution of compound 53 (1.34 g, 3.85 mmol) in DMF (5 ml) was added in small portions. The mixture was stirred at 75 °C for 2 h. The solvent was distilled off, the residue was taken up in chloroform, washed with a saturated solution of ammonium chloride, dried (Na_2SO_4) and evaporated. The oily residue was purified by column chromatography (hexane - ethyl acetate 8:2) which gave compound 54 (1.22 g, 47%). $^1\text{H-NMR}$ (CDCl_3): δ 1.98 (t, 3H), 1.20-2.05 (9H), 2.20 (s, 6H), 2.55, 2.65 (s, 12H), 2.70-3.55 (9H), 6.85 (s, 4H); $^{13}\text{C-NMR}$ (CDCl_3): δ 12.49, 20.80, 21.64, 21.87, 22.88, 28.72, 33.16, 36.13, 39.96, 43.80, 47.95, 57.77, 61.26, 131.83, 132.94, 133.14, 138.82, 139.90, 142.07, 142.63. MS-FAB (m/z) 628 ($\text{M}^+ + 1$), 546 ($\text{M}^+ - 81$).

Compound 55 was obtained following the procedure described for compound 42. From 1.22 g (1.6 mmol) of bromoderivative 54 and 820 mg (1.76 mmol) of diamide 46, 1.26 g (77%) of tetramide 55 was obtained as a glassy oil. $^1\text{H-NMR}$ (CDCl_3): δ 0.80 (t, 6H), 1.20-1.75 (6H), 1.90 (m, 1H), 2.15 (s, 12H), 2.35-2.60 (s, 24H), 2.65-3.40 (15H), 6.85 (b, 8H); $^{13}\text{C-NMR}$ (CDCl_3): δ 12.38, 20.71, 22.52, 22.66, 24.72, 27.55, 28.04, 39.19, 39.71, 41.02, 42.33, 42.62, 43.37, 48.81, 61.44, 131.76, 131.88, 133.10, 133.89, 138.66, 139.93, 142.17, 142.33, 142.57. MS-FAB (m/z) 1012 (M^+), 828 ($\text{M}^+ - 184$).

Compound 56 was obtained following the procedure described for compound 43. From 1.26 g (1.24 mmol) of tetramide 55, 300 mg (56%) of the tetrahydrochloride 56 was obtained; mp > 270°C (decomp). ¹H-NMR (D₂O): δ 1.35 (t, 6H), 1.60 (m, 1H), 1.80 (b, 3H), 2.15 (b, 6H), 2.50 (b, 1H), 3.20 (m, 13H), 3.45 (m, 2H); ¹³C-NMR (D₂O): δ 13.23, 25.48, 25.73, 25.79, 31.69, 31.99, 43.40, 45.91, 46.43, 46.71, 48.07, 53.20, 75.28. MS-MALDI (m/z) 285 (M⁺ + 1).

Compound 57: NaH (80%, 150 mg, 5 mmol) and NaBr (2.5 g, 25 mmol) were added to a solution of compound 52 (2.35 g, 4.9 mmol) in DMF (15 ml). The mixture was stirred at 20°C for 30 min and a solution of 1-bromoethane (2.2 g, 25 mmol) in DMF (10 ml) was added in small portions. The mixture was stirred at 90°C for 3 h. The solvent was distilled off, the residue taken up in chloroform, washed with a saturated solution of ammonium chloride, dried (Na₂SO₄) and evaporated. The product was purified by silica gel chromatography (hexane/ethyl acetate 9:1). The oily residue (1.5 g, 79%) crystallized on standing; mp 68-69°C. ¹H-NMR (CDCl₃): δ 1.10 (t, 3H), 1.30-2.10 (6H), 2.25 (b, 4H), 2.60 (s, 6H), 3.20 (m, 2H), 3.35 (m, 2H), 3.60 (m, 2H), 6.95 (s, 2H); ¹³C-NMR (CDCl₃): δ 16.35, 20.93, 21.79, 22.89, 29.32, 29.37, 36.54, 38.12, 44.13, 61.40, 131.99, 132.80, 140.20, 142.52. MS-FAB 389 (M⁺ + 1), 308 (M⁺ - 80).

Compound 59 was obtained following the procedure described for compound 42. From 700 mg (1.80 mmol) of compound 57 and 394 mg (0.9 mmol) of diamide 58, 400 mg (37%) of tetramide 59 were obtained. ¹H-NMR (CDCl₃): δ 0.90 (t, 6H), 1.25-1.80 (m, 8H), 1.80-2.10 (m, 8H), 2.15 (s, 12H), 2.40, 2.50 (s, 24H), 2.60-3.35 (m, 6H), 2.85, 2.90 (s, 8H); ¹³C-NMR (CDCl₃): δ 16.14, 20.85, 21.95, 21.99, 22.55, 25.49, 28.78, 28.88, 31.49, 37.87, 40.50, 40.83, 43.85, 44.06, 49.30, 61.42, 131.86, 131.96, 133.09, 133.40, 139.93, 139.98, 142.27, 142.40. MS-FAB (m/z) 1052 (M[⊕]), 891 (M⁺ - 184).

Compound 60 was obtained following the procedure described for compound 43. From 400 mg (0.38 mmol) of tetramide 59, 95 mg (53%) of the tetrahydrochloride derivative were obtained; mp > 270°C (decomp.) ¹H-NMR (D₂O): δ 1.30 (t, 6H), 1.60 (m, 2H), 1.80 (m, 6H), 1.95-2.35 (6H), 2.45 (m, 2H), 3.20 (m, 10H), 3.40 (m, 4H); ¹³C-NMR (D₂O): δ 13.59, 25.34, 25.71, 31.75, 32.00, 43.34, 44.83, 48.02, 53.24, 64.52. MS-MALDI (m/z) 325 (M⁺ + 1).

Compound 62: Mesitylenesulfonylchloride (3.27 g, 15 mmol) in dioxane (20 ml) was added dropwise to a stirred solution of 61 (1.3 g, 10 mmol) in dioxane (20 ml) and 50% KOH (15 ml) at 0°C. When addition was completed, the mixture was left over night at 20°C. Excess water was added, the solution cooled and the precipitate filtered off.

5 Crystallization from ethylacetate - hexane gave compound 62 (2 g, 80%); mp 115-116°C.
¹H-NMR (CDCl₃): δ 2.35 (s, 3H), 2.55 (t, 2H), 2.65 (s, 6H), 3.25 (q, 2H), 5.15 (t, 1H), 7.0 (s, 2H); ¹³C-NMR (CDCl₃): δ 19.07, 20.82, 22.78, 38.37, 117.56, 132.07, 133.0, 138.99, 142.67. MS-EI (m/z) 252 (M⁺).

10 **Compound 63:** NaH (80%, 330 mg, 11 mmol) was added to a solution of compound 62 (2.52 g, 10 mmol) in DMF (20 ml) under N₂. The mixture was stirred for 30 min and a solution of compound 53 (3.82 g, 11 mmol) in DMF (10 ml) was added in small portions. The mixture was stirred at 70°C for 2h. The solvent was distilled off, the residue taken up in chloroform, washed with a saturated solution of ammonium chloride, dried (Na₂SO₄) and evaporated to dryness. The product was purified by silica-gel chromatography (hexane - ethyl acetate 8:2). The oily residue (3.0 g, 57%) crystallized on standing; mp 105-106°C.
15 ¹H-NMR (CDCl₃): δ 1.00 (t, 3H), 1.75 (m, 2H), 2.35 (s, 6H), 2.60 (14H), 3.10 (m, 6H), 3.45 (t, 3H), 6.90, 6.95 (s, 4H); ¹³C-NMR (CDCl₃): δ 12.63, 16.94, 20.89, 22.67, 25.73, 40.27, 42.19, 42.51, 44.72, 117.36, 131.95, 132.22, 140.06, 140.34, 142.52, 143.33. MS-EI (m/z) 519 (M⁺), 429 (M⁺ - HCN).

20 **Compound 65:** The nitrile 63 (3.0 g, 5.7 mmol) was dissolved in a mixture of ethanol (150 ml) and concentrated hydrochloric acid (1.5 ml). PtO₂ was added (300 mg), the mixture was hydrogenated at 50 psi over night, the catalyst was filtered off and the solvent evaporated to afford an oily residue of compound 64, which was used in the next step without further purification. Free base ¹H-NMR (CDCl₃): δ 1.00 (t, 3H), 1.55 (m, 2H), 1.75 (m, 2H), 2.30 (s, 6H), 2.55 (14 H), 2.90-3.30 (8H), 6.95 (s, 4H); ¹³C-NMR (CDCl₃): δ 12.64, 20.87, 22.69, 25.35, 30.93, 39.04, 40.12, 42.65, 43.11, 131.86, 133.10, 140.04, 142.43. MS-FAB (m/z) 524 (M⁺ + 1).

25 Mesitylenesulfonylchloride (1.86 g, 8.55 mmol) in dioxane (15 ml) was added dropwise to a stirred mixture of 64 (3.0 g, 5.7 mmol) dissolved in dioxane (30 ml) and 50% KOH (15 ml) at 0°C. The reaction mixture was allowed to reach room temperature and was kept for further 2 h. An excess of water was added and the mixture was extracted with chloroform, dried (Na₂SO₄) and evaporated to dryness. Purification was achieved by silica gel column

chromatography using hexane - ethyl acetate (8:2) as eluant; 2.79 g (69%) of 65 were obtained. ¹H-NMR (CDCl₃): δ 0.95 (t, 3H), 1.60 (m, 4H), 2.30 (s, 9H), 2.50 (s, 12H), 2.65 (s, 6H), 2.85 (m, 2H), 3.05 (6H), 3.20 (t, 2H), 5.00 (t, 1H), 6.95 (6H); ¹³C-NMR (CDCl₃): δ 12.45, 20.81, 22.73, 25.23, 27.46, 39.19, 33.99, 42.49, 42.92, 43.17, 131.84, 133.05, 133.82, 138.80, 139.90, 141.92, 142.36, 142.64. MS-FAB (m/z) 705 (M[⊕]).

Compound 66 was obtained following the procedure described for compound 42. From 705 mg (1 mmol) of 65 and 426 mg (1.1 mmol) of 57, 470 mg (46%) of tetramide 66 was obtained as a glassy product. ¹H-NMR (CDCl₃): δ 0.85-1.10 (t, 6H), 1.35-2.10 (m, 11H), 2.30 (s, 12H), 2.40-2.65 (m, 24H), 2.75-3.55 (m, 13H), 6.95 (m, 8H); ¹³C-NMR (CDCl₃): δ 12.64, 16.11, 20.91, 22.08, 22.75, 24.81, 25.09, 28.83, 29.07, 37.93, 40.08, 40.84, 42.50, 42.81, 43.11, 43.42, 49.11, 61.43. MS-FAB (m/z) 1013 (M⁺ + 1).

Compound 67 was obtained following the procedure described for compound 43. From 470 mg (0.46 mmol) of tetramide 66, 142 mg (71%) of the tetrahydrochloride derivative was obtained; mp >250 °C (decomp). ¹H-NMR (D₂O): δ 1.30 (t, 6H), 1.60 (m, 1H), 1.85 (b,s, 3H), 2.15 (m, 6H), 2.45 (m, 1H), 3.15 (m, 13H), 3.45 (m, 2H); ¹³C-NMR (D₂O): δ 13.29, 13.57, 25.34, 25.44, 25.64, 31.68, 31.94, 43.27, 44.80, 45.86, 46.62, 47.42, 47.97, 53.19, 64.50. MS-MALDI 285 (M⁺ + 1), 286 (M⁺ + 2).

Compound 68a: 4-Cyanobenzaldehyde (Aldrich, 1.31 g, 10 mmol) was dissolved in 30 ml anhydrous MeOH followed by the addition of MgSO₄ (anhydrous, 1.5 g) and 1,4-diaminobutane (Aldrich, 0.44 g, 5 mmol) and the mixture was stirred under argon over night. The suspension was cooled in an ice bath and NaBH₄ (2.0 g) was added in portions and stirring continued for 2 h at 0°C. The methanol was evaporated under vacuum and the resulting solid was partitioned between 35 ml H₂O and 50 ml CHCl₃. Some of the solid was not soluble in either the H₂O or the CHCl₃ and was filtered off and the aqueous layer was extracted with CHCl₃ (2 x 25 ml). The pooled organic layers were dried (MgSO₄), evaporated and the solid was recrystallized from ethyl acetate - hexane, yield 1.1 g (35%); mp 90.6-90.8°C. ¹H-NMR (CDCl₃): δ 1.43 (broad, 2H, NH), 1.55 (m, 4H, CH₂), 2.63 (m, 4H, NCH₂), 3.85 (s, 4H, benzylic CH₂), 7.44 (m, 4H, Ph), 7.60 (m, 4H, Ph); ¹³C-NMR (CDCl₃): δ 27.78, 49.28, 53.44, 110.65, 118.88, 128.52, 132.12, 146.21. MS (m/z) 318 (M⁺), 185, 145, 131, 116 (100%), 70.

Compound 68b was prepared from 4-cyano-benzaldehyde and 1,5-diaminopentane as described above for 68a; 42% yield; mp 92.9-93.0°C. ¹H-NMR (CDCl₃): δ 1.40 (m, 4H, NH, CH₂), 1.50 (m, 4H, CH₂), 2.59 (m, 4H, NCH₂), 3.83 (s, 4H, benzylic CH₂), 7.45 (m, 4H, Ph), 7.59 (m, 4H, Ph); ¹³C-NMR (CDCl₃): δ 24.86, 29.87, 49.29, 53.40, 110.50, 118.85, 128.48, 132.04, 146.19. MS (m/z) 332 (M⁺), 216, 199, 145, 116 (100%), 84.

5 **Compound 68c** was prepared from 4-cyanobenzyldehyde and 1,6-diaminohexane as described above for 68a; 45% yield; mp 95.6-95.8°C. ¹H-NMR (CDCl₃): δ 1.35 (m, 4H, CH₂), 1.50 (m, 6H, NH, CH₂), 2.60 (t, J=6.92 Hz, 4H, NCH₂), 3.84 (s, 4H, benzylic CH₂), 7.44 (m, 4H, Ph), 7.60 (m, 4H, Ph); ¹³C-NMR (CDCl₃): δ 27.17, 30.02, 49.42, 53.50, 10.65, 118.92, 128.55, 132.14, 146.27. MS (m/z) 346 (M⁺), 230, 213, 145, 116 (100%) 98.

10 **Compound 69a:** Dinitrile 68a (0.75 g, 2.36 mmol) was dissolved in anhydrous THF, lithium bis(trimethylsilyl)amide (9.43 ml of a 1 m solution in THF) was added slowly under argon atmosphere. The mixture was stirred at room temperature for 2 h; then cooled in an ice bath, followed by the addition of 4 equivalents of 6N HCl in ether. A white solid precipitated immediately and was filtered after 12 h. The solid was recrystallized from ethanol-ether to afford 1.19 g of compound 69a (93%). ¹H-NMR (D₂O): δ 1.87 (m, 4H, CH₂), 3.22 (m, 4H, CH₂N), 4.40 (s, 4H, benzylic CH₂), 7.74 (m, 4H, Ph), 7.91 (m, 4H, Ph); ¹³C-NMR (DMSO-d₆): δ 22.68, 46.09, 49.28, 128.10, 128.47, 130.69, 138.15, 165.44. 20 MS-ESI (m/z) 353.2 (M⁺), 177.2 (100%).

15 **Compound 69b** was prepared from 68b in 92% yield as described above for 69a. ¹H-NMR (D₂O): δ 1.52 (m, 2H, CH₂), 1.80 (m, 4H, CH₂), 3.19 (m, 4H, NCH₂), 4.40 (s, 4H, benzylic CH₂), 7.75 (m, 4H, Ph), 7.91 (m, 4H, Ph); ¹³C-NMR (DMSO-d₆): δ 24.90, 26.91, 48.96, 51.88, 130.29, 130.46, 132.43, 139.51, 167.52. MS-ESI (m/z) 367.2 (M⁺), 350.2 (100%), 301.2.

20 **Compound 69c** was prepared from 68c as described above for 69a in 96% yield. ¹H-NMR (D₂O): δ 1.46 (m, 4H, CH₂), 1.78 (m, 4H, CH₂), 3.16 (m, 4H, NCH₂), 4.39 (s, 4H, benzylic CH₂), 7.74 (m, 4H, Ph), 7.91 (m, 4H, Ph); ¹³C-NMR (DMSO-d₆): δ 25.24, 25.82, 46.73, 49.44, 128.35, 128.56, 130.81, 138.38, 165.58. MS-ESI (m/z) 381.2 (M⁺), 191.2 (100%), 30 150, 116.

Compound 70: Triamide 18 (4.3 g, 5.8 mmol) was dissolved in 30 ml of DMF and 80% NaH (208 mg, 6.9 mmol) was added. The mixture was stirred under a N₂ atmosphere for 1 h and 1.12 g (7.5 mmol) of bromobutyronitrile dissolved in 3 ml of DMF were added all at once. The reaction mixture was heated for 3 h at 90°C. The solvent was distilled-off and the residue was dissolved in chloroform and washed twice with a saturated solution of ammonium chloride; dried (NaSO₄) and evaporated to dryness. Flash chromatography of the residue using hexane – ethyl acetate (6:4) as eluant gave the yellow oil 70 (3.7 g, 77%).

¹H-NMR (CDCl₃): δ 0.95 (t, 3H), 1.35 (m, 8H), 1.85 (m, 2H), 2.20 (t, 2H), 2.30 (s, 9H), 2.55 (s, 18H), 3.10 (m, 10H), 3.25 (t, 2H), 6.95 (s, 6H). MS-FAB (m/z) 823 (M⁺ + Na), 639, 457.

Compound 71: Nitrile 70 (3.7 g, 4.6 mmol) was dissolved in 20 ml of chloroform and 150 ml of ethanol were added. The mixture was reduced over 0.35 g of PtO₂ at 50 psi over night. The catalyst was filtered-off and the solvent evaporated to dryness. The oily residue was dried in vacuo for 2 h and dissolved in 50 ml of Cl₃CH and 12 ml 2N NaOH. The mixture was cooled in an icewater bath with efficient magnetic stirring and 1.50 g (6.9 mmol) of mesitylene chloride dissolved in 10 ml of chloroform were added all at once. After 2 h the organic layer was separated, washed twice with a saturated solution of ammonium chloride, dried (NaSO₄) and evaporated to dryness. Flash chromatography of the residue using hexane – ethyl acetate (7:3) as eluant provided the tetramide 71 as a colorless oil (3.3 g, 73% over two steps). ¹H-NMR (CDCl₃): δ 0.95 (t, 3H), 1.40 (m, 12H), 2.30 (s, 12H), 2.60 (s, 24H), 2.80 (b, 2H), 3.10 (m, 12H), 4.70 (b, 1H), 6.90 (s, 8H). MS-FAB (m/z) 1010 (M⁺ + 1 + Na), 826, 643.

Compound 72: The tetramide 71 (6.28 g, 6.3 mmol) was dissolved in 40 ml of DMF and 80% NaH (230 mg, 7.6 mmol) was added. The mixture was stirred under a N₂ atmosphere for 1 h and 1.30 g (8.8 mmol) of bromobutyronitrile dissolved in 3 ml of DMF were added all at once. The reaction mixture was heated for 3h at 90°C, the solvent was distilled-off and the residue was extracted into chloroform and washed twice with a saturated solution of ammonium chloride; dried (NaSO₄) and evaporated to dryness. Flash chromatography of the residue with hexane – ethyl acetate (7:3) as eluant provided the nitrile 72 (5.0 g, 74%). ¹H-NMR (CDCl₃): δ 0.95 (t, 3H), 1.35 (m, 12H), 1.80 (m, 2H), 2.25 (t, 2H), 2.35 (s, 12H), 2.70 (s, 24H), 3.10 (m, 14H), 3.25 (t, 2H), 7.0 (s, 8H). MS-FAB (m/z) 1077 (M⁺ + 1 + Na), 893, 711, 586.

Compound 73: Nitrile 72 (6.0 g, 5.6 mmol) was dissolved in 20 ml of chloroform and 150 ml of ethanol were added. The mixture was reduced over 600 mg of PtO₂ at 50 psi overnight. The catalyst was filtered-off and the solvent evaporated to dryness. The oily residue was dried in vacuo for 2 h and dissolved in 100 ml of chloroform and 15 ml 2N NaOH. The mixture was cooled in an icewater bath with efficient magnetic stirring, and 1.80 g (8.4 mmol) of mesitylene chloride dissolved in 10 ml of Cl₃CH was added all at once. After 2h the organic layer was separated, washed twice with a saturated solution of ammonium chloride, dried (Na₂SO₄) and evaporated to dryness. Flash chromatography of the residue using hexane – ethyl acetate (7:3) as eluant gave the pentamide 73 as a colorless oil (5.0 g, 71% over two steps). ¹H-NMR (CDCl₃): δ 0.95 (t, 3H), 1.35 (m, 16H), 2.30 (s, 15H), 2.55 (s, 30H), 2.75 (bs, 2H), 3.05 (m, 16H), 4.70 (b, 1H), 6.90 (s, 10H). MS-FAB (m/z) 1261 (M⁺ - 1 + Na), 1077, 895.

Compound 74: Pentamide 73 (3.4 g, 2.7 mmol) was dissolved in 30 ml of DMF and 60% NaH (162 mg, 4.05 mmol) was added. The mixture was stirred under a N₂ atmosphere for 0.5 h and 2.3 g (10.8 mmol) of 2-bromoethanol benzylether dissolved in 3 ml of DMF were added all at once. The reaction mixture was heated for 2 h at 80°C, the solvent was distilled-off and the residue was dissolved in chloroform and washed twice with a saturated solution of ammonium chloride, dried (Na₂SO₄) and evaporated to dryness. Flash chromatography of the residue using hexane – ethyl acetate (7:3) as eluant provided the product 74 (2.6 g, 70%). ¹H-NMR (CDCl₃): δ 0.95 (t, 3H), 1.30 (m, 16H), 2.30 (s, 15H), 2.50 (s, 30H), 2.90-3.20 (m, 18H), 3.25 (t, 2H), 2.35 (t, 2H), 4.35 (s, 2H), 6.95 (s, 10H), 7.20-7.35 (m, 5H). ¹³C NMR (CDCl₃): δ 12.65, 20.84, 22.67, 22.71, 24.41, 24.66, 39.97, 44.48, 44.88, 46.59, 68.01, 72.95, 127.46, 127.57, 128.25, 131.83, 131.89, 133.28, 139.88, 139.95, 140.04, 142.16, 142.23. MS-FAB (m/z) 1394 (M⁺ - 2 + Na) 1030.

Compound 75: Pentamide 74 (1.2 g, 0.87 mmol) was dissolved in 12 ml of methylene chloride followed by the addition of 30% HBr/acetic acid (16 ml) and phenol (3.0 g, 32 mmol). The mixture was stirred at room temperature overnight, water (16 ml) was added, followed by extraction with methylene chloride (3 × 10 ml). The aqueous layer was evaporated in vacuo. The residue was dissolved in 2N NaOH (4 ml) and 50% KOH (4 ml) followed by extraction with chloroform (4 × 10 ml). After removal of the solvent the residue was dissolved in ethanol (20 ml) and acidified with concentrated hydrochloric acid (0.5 ml). The white precipitate (75) was recrystallized from aqueous ethanol (440 mg,

90%); mp above 270°C (decomp). $^1\text{H-NMR}$ (D_2O): δ 1.30 (t, 3H), 1.75 (b, 16H), 2.90-3.30 (m, 20H), 2.85 (t, 2H). $^{13}\text{C NMR}$ (D_2O): δ 13.29, 25.48, 25.59, 45.70, 49.04, 49.49, 49.67, 51.88, 59.39. MS-MALDI (m/z) 374 ($\text{M}^+ + 1$).

Compound 76: Pentamide 73 (850 mg, 0.68 mmol) was dissolved in DMF (15 ml) and 5 80% NaH (30 mg, 1 mmol) was added. The mixture was stirred under a N_2 atmosphere at room temperature for 0.5 h and 137 mg (0.30 mmol) of 73 dissolved in 5 ml of DMF were slowly added. The reaction mixture was heated for 2 h at 80°C, the solvent was distilled-off and the residue was dissolved in chloroform and washed twice with a saturated solution of ammonium chloride, dried (NaSO_4) and evaporated to dryness. Flash chromatography of 10 the residue using hexane – ethyl acetate – methanol (6:4:0.1) as eluant afforded the product 76 (590 mg, 77%). $^1\text{H-NMR}$ (CDCl_3): δ 0.95 (t, 6H), 1.15-1.40 (m, 32H), 2.30 (s, 30H), 2.55 (s, 60H), 2.90-3.25 (m, 36H), 3.60 (d, 4H), 5.40 (t, 2H), 6.95 (s, 20H). MS-FAB 2553 ($\text{M}^+ + \text{Na}$).

Compound 77 was obtained following the procedure described for compound 75. From 15 650 mg (0.25 mmol) of decamide 76, 225 mg (81%) of decahydrochloride 77 was obtained; mp > 270°C (decomp). $^1\text{H-NMR}$ (D_2O): δ 1.30 (t, 6H), 1.75 (b, 32H), 3.10 (b, 36H), 3.75 (b, 4H), 6.05 (b, 2H); $^{13}\text{C NMR}$ (D_2O): δ 13.28, 25.57, 45.66, 49.00, 49.13, 49.64, 50.86, 131.15. MS-ESI 711 ($\text{M}^+ + 1$).

Compound 78 was obtained following the procedure described for compound 76. From 20 850 mg of 73, 360 mg (47%) of decamide 78 were obtained. $^1\text{H-NMR}$ (CDCl_3): δ 0.95 (t, 6H), 1.15-1.45 (m, 32H), 2.30 (s, 30H), 2.55 (s, 60H), 2.90-3.20 (b, 36H), 3.65 (d, 4H), 5.40 (t, 2H), 6.90 (s, 20H). MS-FAB (m/z) 2553 ($\text{M}^+ + \text{Na}$).

Compound 79 was obtained following the procedure described for compound 75. From 25 330 mg (0.13 mmol) of decamide 78, 127 mg (90%) of decahydrochloride 79 was obtained; mp > 270°C (decomp). $^1\text{H-NMR}$ (D_2O): δ 1.30 (t, 6H), 1.80 (b, s, 32H), 3.10 (b, 36H), 3.85 (d, 4H), 6.0 (t, 2H). $^{13}\text{C NMR}$ (D_2O): δ 13.31, 25.59, 45.71, 46.83, 49.05, 49.39, 49.69, 129.21. MS-ESI (m/z) 512 ($\text{M}^+ + 2$).

Compound 96: A suspension of NaH (60% in mineral oil, 336 mg, 14 mmol) in DMF (50 ml) was slowly added to a stirred solution of benzyl-4-bromobuty ether (3.645 g, 15 mmol) and mesitylenesulfonamide 18 (7.33g, 10 mmol) in DMF (180 ml) at 0°C. The reaction 30 mixture was stirred for 10 h at 50°C, quenched with 15 ml of H_2O at 0°C, acidified to

pH=7 with 5% HCl, extracted with Et₂O, washed with brine, dried (Na₂SO₄), and purified on a column (SiO₂, EtOAc/Hexane = 3:7); yield 6.9 g, (77%). ¹H-NMR (CDCl₃): δ 0.98 (t, J=7.1 Hz, 3H, CH₃), 1.25-1.30 (m, 12H, CH₂), 2.27 (s, 3H, CH₃), 2.29 (s, 6H, CH₃), 2.55 (s, 18H, CH₃), 3.0-3.2 (m, 12H, CH₂), 3.31 (t, J=6.0 Hz, CH₂), 4.41 (s, 2H, CH₂), 6.91 (s, 2H, Ph), 6.92 (s, 4H, Ph), 7.2-7.4 (m, 5H, Ph).

Compound 97: A solution of HBr 30% in AcOH (45 ml) was added to a stirred solution of 96 (2 g, 2.24 mmol) and phenole (6.324 g, 67.2 mmol) in CH₂Cl₂ (23 ml) at 0°C. The cooling bath was removed and the reaction mixture was stirred for 24 h at 20°C. The reaction mixture was quenched with H₂O (45 ml), washed with CH₂Cl₂, and concentrated to dryness *in vacuo*. The residue was cooled to 0°C, basified with 2N NaOH (5 ml), followed by 50% KOH (5 ml). The product was extracted with CHCl₃ (7 x 10 ml); yield 475 mg (81%). ¹H-NMR (D₂O): δ 1.10 (t, J=7 Hz, 3H, CH₃), 1.45-1.70 (m, 12H, CH₂), 2.55-2.70 (m, 12H, CH₂), 3.57 (t, J=5.0 Hz, CH₂).

Compound 98: A solution of Na₂CO₃ (10%, 26 ml) was added to triamine 97 (830 mg, 3.20 mmol) in dioxane (21 ml). Di-*tert*-butyl dicarbonate (3.25 g, 24 mmol) solution in dioxane (21 ml) was added into the reaction mixture at 0° and stirred for 10 h at 20°C. The reaction mixture was diluted with CHCl₃ (200 ml), washed with H₂O, brine, dried (Na₂SO₄), concentrated and purified on a column (SiO₂, EtOAc/hexane=4:6); yield 1.7 g, (96%). ¹H-NMR (CDCl₃): δ 1.09 (t, J=7.1 Hz, 3H, CH₃), 1.45-1.65 (m, 39H, CH₂, CH₃), 3.1-3.3 (m, 12H, CH₂), 3.67 (t, J=6 Hz, CH₂).

Compound 99: Diethyl azodicarboxylate (196 mg, 1.13 mmol) in THF (0.6 ml) was added to a cold mixture of 98 (630 mg, 1.127 mmol), triphenylphosphine (296 mg, 1.13 mmol) and phthalimide (166 mg, 1.13 mmol) in THF (1.2 ml), stirred for 10 h, concentrated *in vacuo*, and purified on a column (SiO₂, EtOAc/hexane=3:7); yield 835 mg (97%). ¹H-NMR (CDCl₃): δ 1.09 (t, J=7, 3H, CH₃), 1.35-1.80 (m, 39H, CH₂, CH₃), 3.1-3.35 (m, 12H, CH₂), 3.70 (t, J=6.7 Hz, 2H, CH₂), 7.68-7.80 (m, 2H, Phth), 7.80-7.87 (m, 2H, Phth).

Compound 100: A mixture of 99 (275 mg, 0.4 mmol), hydrazine monohydrate (98%, 43.5 μl, 0.85 mmol) in EtOH were heated at 80°C for 45 min. The precipitate was filtered and washed with cold EtOH, filtrate was combined with washings, concentrated and dried *in vacuo*; yield 220 mg (99%). ¹H-NMR (CDCl₃): δ 1.09 (t, J=7.0 Hz, 3H, CH₃), 1.25-1.57 (m 39H, CH₂, CH₃), 2.71 (t, J=6.7 Hz, 2H, CH₂), 3.1-3.3 (m, 12H, CH₂).

Compound 101 Mesoporphyrin IX dihydrochloride (70 mg, 0.11 mmol) was dissolved in DMF (1 ml), combined with HBTU (83 mg, 0.22 mmol) and diisopropylethyl amine (174 μ l, 1 mmol). The mixture was stirred for 5 min, combined with 100 (167 mg, 0.3 mmol), stirred for 10 h, quenched with H₂O, concentrated *in vacuo*, dissolved in CHCl₃, washed with H₂O (5 times), 2% KHSO₄, NaHCO₃ solution, dried (Na₂SO₄), and purified by a column chromatography (SiO₂, MeOH/CHCl₃ = 1:20); yield 177 mg (98%). ¹H-NMR (CDCl₃): δ 1.06 (t, J=6.9, 6H, CH₃), 1.1-1.5 (m, 78H, CH₂, CH₃), 1.86 (t, J=6.6 Hz, 6H, CH₃), 2.9-3.25 (m, 28H, CH₂), 3.62 (s, 3H, CH₃), 3.63 (s, 3H, CH₃), 3.64 (s, 3H, CH₃), 3.65 (s, 3H, CH₃), 4.07 (q, J=7.5 Hz, 4H, CH₂), 4.43 (t, J=7Hz, 4H, CH₂), 10.07 (s, 3H, CH), 10.27 (s, 1H).

Compound 102: Trifluoroacetate (1 ml) was added to a solution of 101 (165 mg, 0.1 mmol) in CH₂Cl₂ (2 ml) at 0° C, stirred for 1.5 h, and concentrated *in vacuo* at 20° C. The product was dried *in vacuo* for 3h, dissolved in 10 ml of 10% HCl, washed with CHCl₃, and the aqueous part was concentrated and dried *in vacuo* at 20° C; yield 100 mg (75%), purple solid, mp >200° C. Purity 96% (HPLC). ¹H-NMR (D₂O): δ 0.7-1.0 (m), 1.0-1.2 (m), 1.29 (t, J=7.3 Hz), 1.4-1.7 (m), 2.6-2.35 (m), 2.72 (t, J=7.2 Hz), 2.9-3.2 (m), 3.6-3.75 (m), 3.76 (s), 3.79 (s), 4.29 (q, J=7.6 Hz), 4.4-4.7 (m). MS-MALDI (m/z): 1193 (M⁺ + 4 HCl), 1156 (M⁺ + 3 HCl), 1119 (M⁺ + 2 HCl), 1083 (M⁺ + HCl), 1048 (M⁺ + 1).

Compound 103 was prepared from 71 in 85% yield as described above for 96. ¹H-NMR (CDCl₃): δ 0.97 (t, J=7 Hz, 3H, CH₃), 1.3-1.55 (m, 16H, CH₂), 2.27 (s, 3H, CH₃), 2.29 (s, 9H, CH₃), 2.55 (s, 24H, CH₃), 3.0-3.25 (m, 16H, CH₂), 3.31 (t, J=5.8 Hz, 2H, CH₂), 4.41 (s, 2H, CH₂), 6.90 (s, 2H, Ph), 6.92 (s, 6H, Ph).

Compound 104 was prepared from 103 in 80% yield as described for 97. ¹H-NMR (CDCl₃): δ 1.10 (t, J=7.2 Hz, 3H, CH₃), 1.5-1.8 (m, 16H, CH₂), 2.5-2.75 (m, 16H, CH₂), 3.57 (t, J=5.5 Hz, 2H, CH₂). ¹³C-NMR (CDCl₃): δ 15.23, 27.55, 27.92, 28.58, 32.35, 44.02, 49.35, 49.66, 49.80, 62.32.

Compound 105 was prepared from 104 in 98% yield as described for 98. ¹H-NMR (CDCl₃): δ 1.09 (t, J=7.1 Hz, 3H, CH₃), 1.4-1.7 (m, 52 H, CH₂, CH₃), 3.05-3.3 (m, 16H, CH₂), 3.67 (t, J=5.8 Hz, 2H, CH₂).

Compound 106: Oxalyl chloride (2N solution in CH₂Cl₂, 0.821 µl, 1.642 mmol) was diluted with anhydrous CH₂Cl₂ (6 ml) at -60° C. DMSO (223 µl, 2.59 mmol) in CH₂Cl₂ (3 ml) was added to the mixture, the latter stirred for 5 min at -60° C, and 105 (1.115 g, 1.525 mmol) in CH₂Cl₂ (9 ml) was introduced into the reaction. After 30 min of stirring at -60° C
5 triethylamine (1.06 ml, 14.46 mmol) was added into the reaction mixture and the temperature was allowed to rise to 20° C (ca. 1.5 h). The reaction mixture was diluted with CH₂Cl₂, washed with H₂O, NaHCO₃, and brine. The product was concentrated to dryness *in vacuo* and purified by column chromatography (SiO₂, EtOAc/Hexane= 3:7); yield 989 mg (89%). ¹H-NMR (CDCl₃): δ 1.09 (t, J=7.0 Hz, 3H, CH₃), 1.4-1.6 (m, 48H, CH₂, CH₃),
10 1.84 (m, 2H, CH₂), 2.45 (t, J=6.8, 2H, CH₂), 3.05-3.3 (m, 16H, CH₂), 9.78 (s, 1H, CHO).

Compound 107: Platinum oxide (100 mg) was reduced in methanol (30 ml) in the hydrogen atmosphere at 30 psi. for 15 min. Product 106 (989 mg, 1.36 mmol) was dissolved in a 2M solution of ethylamine in EtOH (7 ml), added to the hydrogenation flask, and hydrogenated for 10 h at 50 psi. The catalyst was removed by filtration through Celite and the filtrate was concentrated to dryness *in vacuo*; yield 1.0 g (99%). ¹H-NMR (CDCl₃): δ 1.09 (t, J=7.6 Hz, 3H, CH₃), 1.12 (t, J=7.2 Hz, 3H, CH₃), 1.3-1.65 (m, 50H, CH₂, CH₃),
15 1.66 (m, 2H, CH₂), 2.71 (m, 2H, CH₂), 3.1-3.3 (m, 18H, CH₂). MS-MALDI (m/z): 758.8 (M⁺, 100%), 744 (30%).

20 **Compound 108** was prepared from 107 in 98% yield as described above for 101. ¹H-NMR (CDCl₃): δ 0.8-1.8 (m), 1.87 (t, J=7.3 Hz, 6H, CH₃), 2.5-3.5 (m), 3.65 (s, 6H, CH₃), 3.67 (s, 6H, CH₃), 4.10 (q, J=7.5, 4H, CH₂), 4.46 (t, J=6.8, 4H, CH₂), 10.11 (bs, 4H, porph. core).

Compound 109 was prepared from 108 in 75% yield as described above for 102; purple solid, mp >200° C. Purity 96% (HPLC). ¹H-NMR (D₂O): δ 0.25-0.5 (m), 0.6-0.8 (m), 1.1-1.5 (m), 1.5-2.0 (m), 1.32 (t), 2.6-3.4 (m), 3.6-3.9 (m), 3.78 (s), 3.82 (s), 4.2-4.6 (m). MS-MALDI (m/z): 1246.22 (M⁺ +1), 623.82 (M²⁺).

25 **Compound 111:** Nitrile 70 (274 mg, 0.343 mmol) was hydrogenated in a 5% solution of CHCl₃ in EtOH (20 ml) in the presence of PtO₂ (60 mg) at 50 psi for 15 h. The reaction mixture was filtered through Celite and concentrated to dryness *in vacuo* to yield 110 (288 mg, 100%). Amine 110 was utilized without further purification. Mesoporphyrin IX dihydrochloride (100 mg, 0.156 mmol) was dissolved in DMF (4 ml), HBTU (118 mg, 0.312

mmol) and DIEA (174 μ l, 2 mmol) were added to the solution, the latter stirred for 5 min, combined with 110, and kept for 15 h. The reaction mixture was quenched with 0.5 ml of H₂O, concentrated *in vacuo*, dissolved in CHCl₃, washed with 3% HCl solution, brine, dried (Na₂SO₄), and purified on a column (SiO₂, CHCl₃/MeOH=15:4); yield 327 mg (98%). ¹H NMR (CDCl₃): δ 0.5-0.8 (m), 0.8-1.1 (m), 0.87 (t, J=7 Hz), 1.1-1.3 (m), 1.5-1.8 (m), 1.88 (q, J=5 Hz), 2.0-2.3 (m), 2.10 (s, CH₃-Mes), 2.13 (s, CH₃-Mes), 2.16 (s, CH₃-Mes), 2.18 (s, CH₃-Mes), 2.43 (s, CH₃-Mes), 2.49 (s, CH₃-Mes), 2.7-2.9 (m), 2.9-3.2 (m), 3.64 (s, porph), 3.66 (s, porph), 4.1 (q, porph.), 4.4 (t, porph.), 6.69 (s, NH), 6.80 (s, NH), 6.85 (s, NH), 6.93 (s, NH), 10.07 (s, porph), 10.09 (s, porph), 10.22 (s, porph.). MALDI (m/z): 2141.03 (M⁺ + 1), 1071.22 (M²⁺).

Compound 112: Porphyrin 111 (350 mg, 0.163 mmol) was stirred in a suspension of LiAlH₄ (12.4 mg, 0.326 mmol) in THF (1 ml) for 24 h at 50° C. The reaction mixture was cooled to 0° C, quenched with H₂O (0.1 ml), basified with 2N NaOH (0.5 ml), then diluted with CH₂Cl₂, filtered through Celite, washed with H₂O, dried (Na₂SO₄), and concentrated *in vacuo*. The product was stirred in a mixture of phenol (920 mg, 9.4 mmol) in CH₂Cl₂ (8 ml), and 30% solution of HBr in AcOH (7 ml) for 15 h. The reaction mixture was cooled to 0° C, diluted with 10 ml of H₂O and the aqueous layer was filtered and washed with CH₂Cl₂. Following drying *in vacuo* of the aqueous solution, the residue was basified with 2N NaOH (1 ml) and 16 N NaOH (1 ml) and the reaction product was extracted with CHCl₃. The product 112 was purified by HPLC and converted to its hydrochloride by dissolution in 10% HCl and evaporation of the acid *in vacuo*; purple solid, mp 200° C. Purity 96% (HPLC). ¹H NMR (D₂O): δ 1.32 (t, J=7.3 Hz, 6H, CH₃), 1.25-1.35 (m, 30H, CH₂), 2.5-2.65 (m, 4H, CH₂), 3.0-3.3 (m, 32H, CH₂), 3.4-3.5 (m, 4H, CH₂), 3.55-3.65 (m, 6H, CH₃), 3.73 (s, 3H, CH₃), 3.75 (s, 3H, CH₃), 4.0-4.25 (m, 4H, CH₂), 4.3-4.5 (m, 4H). MALDI (m/z): 1073.8 (M⁺ + NH₄Cl), 1020.0 (M⁺ + 1), 510.62 (M²⁺), 340.82 (M³⁺).

Compound 113: Diisobutylaluminum hydrate (1.16 ml of 1.5 M solution in toluene, 1.74 mmol) was added into a solution of mesoporphyrin IX dimethyl ester (500 mg, 84 mmol) in CH₂Cl₂ (10 ml) at -78° C, stirred at this temperature for 1h, quenched with saturated solution of NH₄Cl (1 ml), followed by 3.7% solution of HCl (2 ml). The temperature of the reaction mixture was allowed to rise to 20° C, the product was extracted with CH₂Cl₂, dried (Na₂SO₄), and purified on a column (SiO₂, EtOAc/Hexane=3:7), yield 330 mg (73%). ¹H-NMR (CDCl₃): δ 1.86 (t, J=7.6 Hz, 6H, CH₃), 3.39 (t, J=7.4 Hz, 6H, CH₃), 3.60 (s, 6H,

CH₃), 3.62 (s, 6H, CH₃), 4.0–4.2 (m, 4H, CH₂), 4.25–4.45 (m, 4H, CH₂), 9.97 (s, 1H), 10.04 (s, 1H), 10.05 (s, 1H), 10.058 (s, 1H), 10.062 (s, 1H), 10.07 (s, 1H).

Table 1. Polyamine analogs

Compound	Structure
SL-11090	<p>.4HCl</p>
SL-11091	<p>.4HCl</p>
SL-11092	<p>.4HCl</p>
SL-11101	<p>.4HCl</p>
SL-11103	<p>.4HCl</p>
SL-11114	<p>4HCl</p>
SL-11118	<p>4HCl</p>
SL-11121	<p>5HCl</p>
SL-11122	<p>5HCl</p>
SL-11123	<p>5HCl</p>

SL-11124	
SL-11126	
SL-11127	
SL-11128	
SL-11129	
SL-11130	
SL-11132	
SL-11133	
SL-11134	
SL-11136	

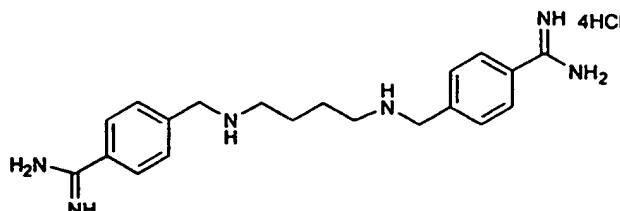
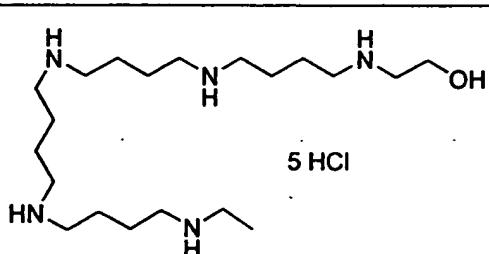
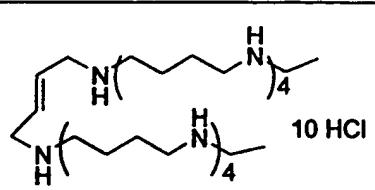
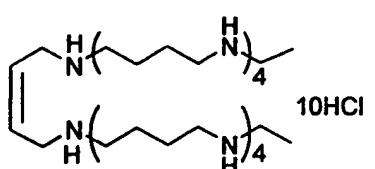
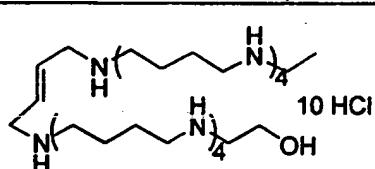
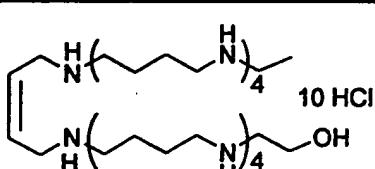
SL-11137	
SL-11141	 5 HCl
SL-11144	 10 HCl
SL-11150	 10HCl
SL-11201	 10 HCl
SL-11202	 10 HCl

Table 2. Polyamine-porphyrin conjugates

SL-11161	
SL-11162	
SL-11164	
SL-11177	
SL-11184	
SL-11202	

5

EXAMPLE 2***In vitro* testing of the efficacy of novel polyamine analogs against tumor cell lines**

These experiments are designed to evaluate the newly synthesized compounds described above against cultured human carcinoma cell lines for their effects on cell growth, cell-cycle regulation and polyamine regulatory responses. Additional methods for testing compounds are described in U.S. Patent No. 5,889,061.

As shown in Table 3 and Figs. 1-32, several novel conformationally restricted polyamine analogs were tested for anti-proliferative properties against cancer cells. Table 3 illustrates the concentration in μM of the various novel polyamine analogs needed for 50% growth inhibition (ID_{50}) values for human cancer cell lines LNCaP, PC-3, DuPro (all three human prostate cancer cell lines), HT-29 (colon cancer cell line), A549 (lung cancer cell line), MCF7 (breast cancer cell line), and U251 MG-NCI (brain cancer cell line). Figs. 1-32 show a representative plot of the effects of some of these novel analogs on the growth of human tumor cell lines, as determined by MTT (methyl thiazol tetrazolium) assay; known anti-proliferative polyamine analogs BE-333, BE-343, BE-444, and BE-4444 were used for comparative purposes.

Cell Lines and Media

Human breast cancer cell line MCF7 was grown in Richter's Improved Modified Eagle's Medium supplemented with 10% fetal bovine serum (FBS) and 2.2 g/L sodium bicarbonate. Human brain tumor cell line U251 MG-NCI was grown in Dulbecco's Modified Eagle's Medium supplemented with 10% FBS. Human lung cancer cell line A549 was grown in Ham's F-12K medium (Cellgro, Mediatech, Inc., VA), supplemented with 10% FBS and 2 mM L-glutamine. Human colon cancer cell line HT29 was cultured in McCoy's 5A medium (Gibco, BRL, Gaithersburg, MD) supplemented with 10% FBS. Human prostate cancer cell lines PC-3, LNCAP and DuPro were grown in RPMI 1640 Medium (Cellgro, Mediatech, Inc., VA) supplemented with 10% FBS. Another human prostate cancer cell line DU145 was grown in Dulbecco's Modified Eagle's Medium (Gibco, BRL, Gaithersburg, MD) supplemented with 5% FBS. The A549, MCF7, PC3, LNCAP and DuPro cell lines were cultured in 100 units/mL penicillin and 100 $\mu\text{g}/\text{mL}$ streptomycin. HT29 and U251MG cell lines were grown in 50 $\mu\text{g}/\text{mL}$ gentamycin (Gibco, BRL, Gaithersburg, MD). DU145 cell line was maintained in 1% antibiotic-antimycotic solution (Sigma, St. Louis, MO). The cell cultures were maintained at 37°C in 5% CO_2 /95% humidified air. DuPro cells were obtained from M. Eileen Dolan, University of Chicago. All other cells are available from the American Type Culture Collection, Rockville, MD.

30

MTT assay

A conventional MTT assay was used to evaluate percent cell survival. Exponentially growing monolayer cells were plated in 96-well plates at a density of 500 cells per well and allowed to grow for 24 hours. Serial dilutions of the drugs were added to the wells. Six days after drug treatment, 25 µl of MTT solution (5 mg/ml) was added to each well and incubated for 4 hours at 37°C. Then 100 µl of lysis buffer (20% sodium dodecyl sulfate, 50% DMF, and 0.8% acetic acid, pH 4.7) was added to each well and incubated for an additional 22 hours. A microplate reader ("EMAX"-brand, Molecular Devices, Sunnyvale, Calif.) set at 570 nm was used to determine the optical density of the cultures. Results are expressed as a ratio of the optical density in drug-treated wells to the optical density in wells treated with vehicle only.

TABLE 3

	PC-3	DU-145	DUPRO	HT-29	A549	MCF7	U251M G
BE-4444	0.54	0.07	0.2	0.8	0.4	>31.25	NT
SL-11029	24.5	0.32	NT	>31.25	>31.25	>31.25	>31.25
SL-11090	>31.25	>31.25	NT	>31.25	>31.25	>31.25	>31.25
SL-11091	>31.25	1.33	NT	>31.25	>31.25	>31.25	>31.25
SL-11092	>31.25	1.7	NT	>31.25	>31.25	>31.25	>31.25
SL-11093	14.3	0.01	0.06	0.40	0.26	0.66	NT
SL-11094	>31.25	12.6	NT	28.8	>31.25	>31.25	>31.25
SL-11098	1.4	0.018	0.08	0.40	0.51	>31.25	0.10
SL-11099	2.5	0.014	0.08	1.00	0.65	26.3	0.11
SL-11100	4.7	0.021	0.29	2.00	2.20	>31.25	0.22
SL-11101	7.7	0.218	0.85	5.20	0.15	>31.25	1.70
SL-11102	>31.25	0.027	0.15	0.73	12.40	>31.25	0.15
SL-11103	>31.25	2.8	NT	29.4	>31.25	>31.25	9.50
SL-	>31.25	9.4	NT	25.8	0.43	>31.25	14.71

11104							
SL-11105	>31.25	1.6	>31.25	25.2	>31.25	>31.25	25.9
SL-11108	2.2	0.13	0.98	2.00	>31.25	>31.25	2.00
SL-11114	0.70	0.135	0.64	3.6	>31.25	NT	NT
SL-11118	1.65	0.05	0.25	0.98	0.21	NT	NT
SL-11119	>31.25	0.08	0.44	0.97	NT	NT	NT
SL-11121	0.52	0.08	0.40	0.80	>31.25	17.0	NT
SL-11122	>31.25	0.80	0.56	0.80	>31.25	>31.25	NT
SL-11123	>31.25	0.51	>31.25	10.42	>31.25	>31.25	NT
SL-11124	>31.25	>31.25	>31.25	>31.25	>31.25	>31.25	NT
SL-11126	0.20	0.51	1.10	1.50	>31.25	0.70	NT
SL-11127	>31.25	0.22	1.3	2.91	NT	NT	NT
SL-11128	0.50	0.14	1.25	1.35	NT	NT	NT
SL-11129	1.70	0.32	NT	NT	NT	NT	NT
SL-11130	>31.25	0.43	NT	NT	NT	NT	NT

NT indicates not tested.

5

EXAMPLE 3

In vivo testing of anti-tumor activity of polyamine analogs

Analogs found to have potent or mechanism-based anti-proliferative activity *in vitro* towards cultured carcinoma cells are evaluated in *in vivo* model systems. The first goal is to determine the relative toxicity of the analogs in non-tumor-bearing animals, such as DBA/2 mice. Groups of three animals each are injected intraperitoneally with increasing concentrations of an analog, beginning at 10 mg/kg. Toxicity, as indicated by morbidity, is

closely monitored over the first 24 hr. A well-characterized polyamine analog, such as BE-333, is used as an internal standard in these studies, since a data base has already been established regarding acute toxicity via a single dose treatment relative to chronic toxicity via a daily x 5 d schedule. Thus, in the case of new analogs, single dose toxicity relative to
5 BE-333 is used to project the range of doses to be used on a daily x 5 d schedule.

After the highest tolerated dosage on a daily x 5 d schedule is deduced, antitumor activity is determined. Tumors are subcutaneously implanted into nude athymic mice by trocar and allowed to reach 100-200 mm³ before initiating treatment by intraperitoneal injection daily x 5 d. Analogs are given in a range between 10 and 200 mg/kg. Analogs
10 are evaluated at three treatment dosages with 10-15 animals per group (a minimum of three from each are used for pharmacodynamic studies, described below). Mice are monitored and weighed twice weekly to determine tumor size and toxicity. Tumor size is determined by multi-directional measurement from which volume in mm³ is calculated. Tumors are followed until median tumor volume of each group reaches 1500 mm³ (i.e., 20% of body
15 weight), at which time the animals are sacrificed. Although the initial anti-tumor studies focused on a daily x 5 d schedule, constant infusion is performed via Alzet pump delivery for 5 days since this schedule dramatically improves the anti-tumor activity of BE-333 against A549 human large cell lung carcinoma. Sharma et al. (1997) *Clin. Cancer Res.* 3:1239-1244. In addition to assessing anti-tumor activity, free analog levels in tumor and
20 normal tissues are determined in test animals.

EXAMPLE 4

In vitro testing of porphyrin-polyamine conjugates

Using the protocols of Example 2, the porphyrin-polyamine conjugates were tested
25 for activity *in vitro* in various cancer cell lines. The results are shown in Table 4 and Figs.
45-49.

Table 4

**Effect of Polyamine Porphyrin Complexes on the Growth of Human Prostate Cell
30 Lines by MTT Assay.**

Compounds	IC ₅₀ (μ M) in different cell lines			
	PC3	DUPRO	LNCAP	DU145
SL-11162	2.8	1.9	4.8	
SL-11177	0.9	0.7	4.8	
SL-11184	1.4	1.5		8.4
SL-11202	1.15	1.3		4.6

EXAMPLE 5

In vivo testing of porphyrin-polyamine conjugates

5 Conjugates found to have potent or mechanism-based anti-proliferative activity *in vitro* towards cultured carcinoma cells are evaluated in *in vivo* model systems. The first goal is to determine the relative toxicity of the conjugates in non-tumor-bearing animals, such as DBA/2 mice. Groups of three animals each are injected intraperitoneally with increasing concentrations of an conjugate, beginning at 10 mg/kg. Toxicity, as indicated by 10 morbidity, is closely monitored over the first 24 hr. A well-characterized compound, such as BE-333, is used as an internal standard in these studies, since a data base has already been established regarding acute toxicity via a single dose treatment relative to chronic toxicity via a daily x 5 d schedule. Thus, in the case of new conjugates, single dose toxicity relative to BE-333 is used to project the range of doses to be used on a daily x 5 d schedule.

15 After the highest tolerated dosage on a daily x 5 d schedule is deduced, antitumor activity is determined. Tumors are subcutaneously implanted into nude athymic mice by trocar and allowed to reach 100-200 mm³ before initiating treatment by intraperitoneal injection daily x 5 d. Conjugates are given in a range between 10 and 200 mg/kg. Conjugates are evaluated at three treatment dosages with 10-15 animals per group (a 20 minimum of three from each are used for pharmacodynamic studies, described below). Mice are monitored and weighed twice weekly to determine tumor size and toxicity. Tumor size is determined by multi-directional measurement from which volume in mm³ is calculated. Tumors are followed until median tumor volume of each group reaches 1500 mm³ (i.e., 20% of body weight), at which time the animals are sacrificed. Although the 25 initial anti-tumor studies focused on a daily x 5 d schedule, constant infusion is performed via Alzet pump delivery for 5 days since this schedule dramatically improves the anti-tumor activity of BE-333 against A549 human large cell lung carcinoma. Sharma et al.

(1997) *Clin. Cancer Res.* 3:1239-1244. In addition to assessing anti-tumor activity, free conjugate levels in tumor and normal tissues are determined in test animals.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is apparent to those skilled in the art that certain minor changes and modifications will be practiced. Therefore, the description and examples should not be construed as limiting the scope of the invention, which is delineated by the appended claims. All references disclosed herein, including U.S. Patent No. 5,889,061, are hereby incorporated by reference in their entirety.

CLAIMS

What is claimed is:

5

1. A conformationally restricted polyamine analog of the formula:

E-NH-B-A-B-NH-B-A-B-NH-B-A-B-NH-B-A-B-NH-E

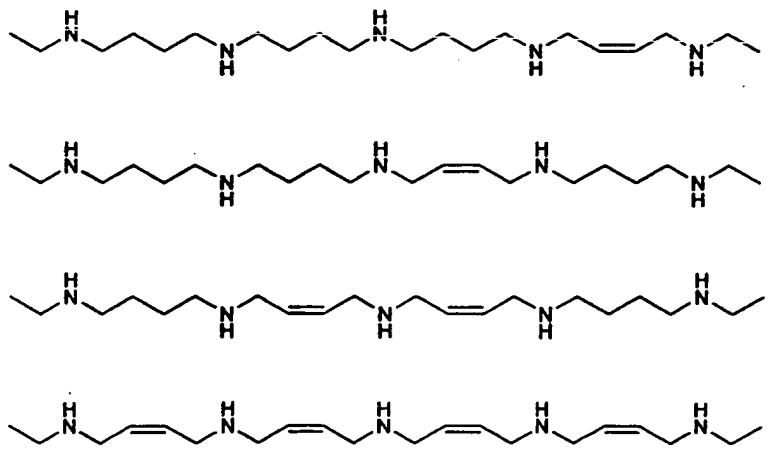
wherein each A is independently selected from the group consisting of: a single bond, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ 10 cycloalkenyl;

each B is independently selected from the group consisting of: a single bond, C₁-C₆ alkyl, and C₂-C₆ alkenyl;

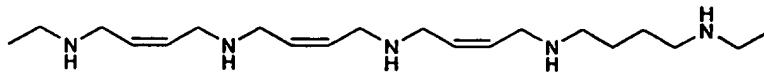
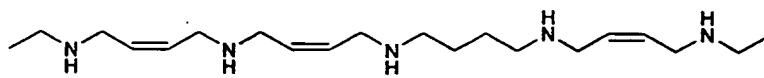
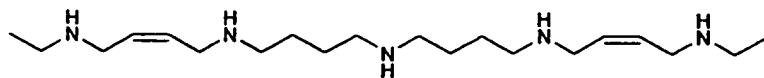
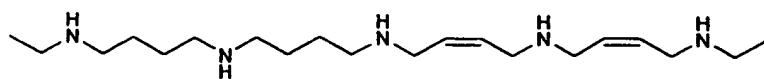
and each E is independently selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl;

15 with the proviso that either at least one A moiety is selected from the group consisting of C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl, or at least one B moiety is selected from the group consisting of C₂-C₆ alkenyl; and any salt or stereoisomer thereof.

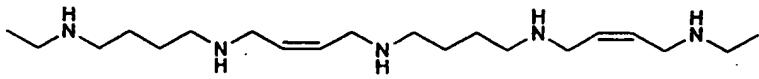
20 2. A conformationally restricted polyamine analog according to claim 1, selected from the group consisting of



25



, and



5

, and any salt or

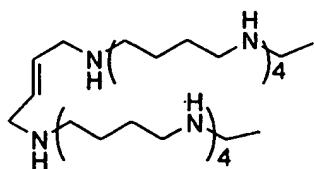
stereoisomer thereof.

3. A conformationally restricted polyamine analog of the formula:

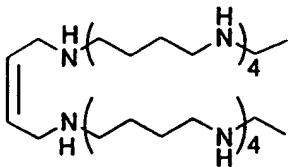


- 10 wherein each A is independently selected from the group consisting of: a single bond, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl;
- each B is independently selected from the group consisting of: a single bond, C₁-C₆ alkyl, and C₂-C₆ alkenyl;
- 15 each E is independently selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl; and x is an integer from 2 to 16;
- with the proviso that either at least one A moiety is selected from the group consisting of C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl,
- 20 or at least one B moiety is selected from the group consisting of C₂-C₆ alkenyl; and any salt or stereoisomer thereof.

4. A conformationally restricted polyamine analog according to claim 3, selected from the group consisting of:



and



;

and any salt or stereoisomer thereof.

5

5. A polyamine analog of the formula:

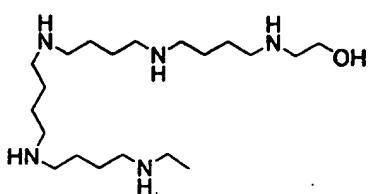
E-NH-B-A-B-NH-B-A-B-NH(-B-A-B-NH)_x-E

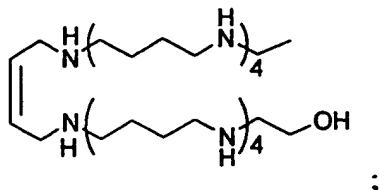
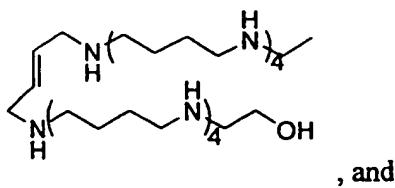
wherein each A is independently selected from the group consisting of: a single bond,

C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl;10 each B is independently selected from the group consisting of: a single bond, C₁-C₆ alkyl, and C₂-C₆ alkenyl;each E is independently selected from the group consisting of C₁-C₆ alkyl, C₁-C₆ alkanol, C₃-C₆ cycloalkanol, and C₃-C₆ hydroxyaryl,with the proviso that at least one E moiety be selected from the group consisting of C₁-C₆ alkanol, C₃-C₆ cycloalkanol, and C₃-C₆ hydroxyaryl;

15 and x is an integer from 0 to 16;

and any salt or stereoisomer thereof.

20 6. A polyamine analog according to claim 5, selected from the group consisting
of:



and all salts and stereoisomers thereof.

5

7. A conformationally restricted polyamine analog of the formula:

E-NH-D-NH-B-A-B-NH-D-NH-E

wherein each A is selected from the group consisting of C₂-C₆ alkynyl;

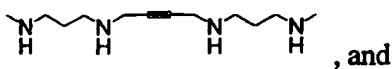
each B is independently selected from the group consisting of: a single bond, C₁-C₆ alkyl, and C₂-C₆ alkenyl;

each D is independently selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloalkenyl, and C₃-C₆ cycloaryl; and
each E is independently selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl;

15 and any salt or stereoisomer thereof.

8. A conformationally restricted polyamine analog according to claim 7, selected from the group consisting of

20





and any salt or stereoisomer thereof.

5 9. A conformationally restricted polyamine analog of the formula:

E-NH-B-A-B-NH-F-NH-B-A-B-NH-E

wherein F is selected from the group consisting of C₁-C₆ alkyl;

each A is independently selected from the group consisting of: a single bond, C₁-C₆ alkyl; C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl;

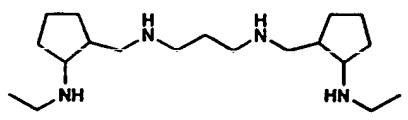
each B is independently selected from the group consisting of: a single bond, C₁-C₆ alkyl, and C₂-C₆ alkenyl; and

each E is independently selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl;

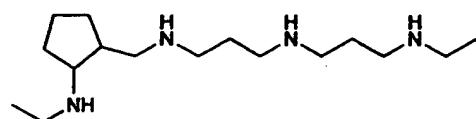
with the proviso that either at least one A moiety is selected from the group consisting of

15 C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl, or at least one B moiety is selected from the group consisting of C₂-C₆ alkenyl; and any salt or stereoisomer thereof.

10. A conformationally restricted polyamine analog according to claim 9, selected from the group consisting of



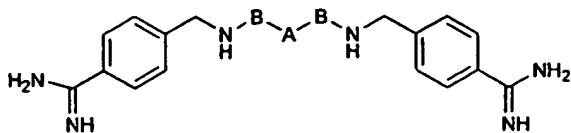
and



• 3

25 and any salt or stereoisomer thereof.

11. A conformationally restricted polyamine analog of the formula:



wherein A is independently selected from the group consisting of: a single bond, C₁-C₆

5 alkyl; C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆

cycloalkenyl;

each B is independently selected from the group consisting of: a single bond, C₁-C₆ alkyl,

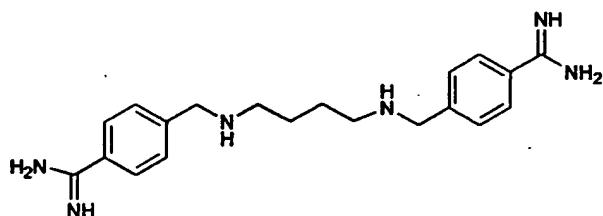
and C₂-C₆ alkenyl;

with the proviso that A and both B moieties are not all a single bond;

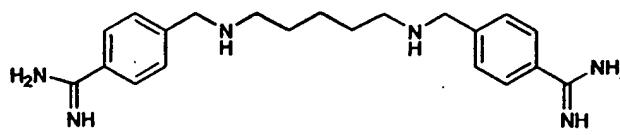
10 and any salt or stereoisomer thereof.

12. A conformationally restricted polyamine analog according to claim 11, selected from

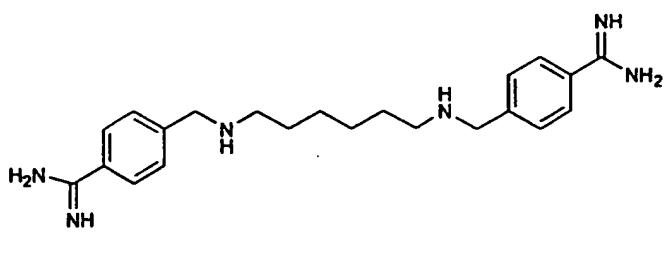
the group consisting of:



15



, and



and any salt or stereoisomer thereof.

5 13. A conformationally restricted polyamine analog of the formula:

E-NH-D-NH-B-A-B-NH-D-NH-E

wherein A is selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆

alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloalkenyl, and C₃-C₆ cycloaryl;

each B is independently selected from the group consisting of: a single bond, C₁-C₆ alkyl,

10 and C₂-C₆ alkenyl;

each D is independently selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl,

C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloalkenyl, and C₃-C₆ cycloaryl; and

each E is independently selected from the group consisting of H and C₁-C₆ alkyl;

with the proviso that either at least one A moiety is selected from the group consisting of

15 C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl, or at least one B moiety is selected from the group consisting of C₂-C₆ alkenyl;

and the proviso that at least one E is selected from the group consisting of H or methyl;

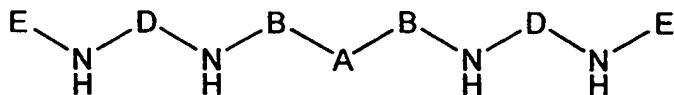
and any salt or stereoisomer thereof.

20 14. A conformationally restricted polyamine analog according to claim 13 of the formula



and any salt or stereoisomer thereof.

15. A conformationally restricted polyamine analog of the formula:



wherein A is *trans*-ethene

each B is independently selected from the group consisting of: a single bond, C₁-C₆ alkyl, 5 and C₂-C₆ alkenyl;

each D is independently selected from the group consisting of C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl, with the proviso that D is not C₃ alkyl; and

each E is independently selected from the group consisting of H, C₁-C₆ alkyl, C₂-C₆ 10 alkenyl, C₂-C₆ alkynyl, C₃-C₆ cycloalkyl, C₃-C₆ cycloaryl, and C₃-C₆ cycloalkenyl;

and any salt thereof.

16. The polyamine analog of claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15, further comprising a pharmaceutically acceptable excipient.

15

17. A method of treating an indication in an individual comprising the step of administering to the individual a therapeutic amount of a polyamine analog of claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15.

20

18. The method of claim 17, wherein the individual is a human.

19. The method of claim 17, wherein the indication is a cancer.

20. The method of claim 19, wherein the cancer affects cells of the bladder, blood, 25 brain, breast, colon, digestive tract, lung, ovaries, pancreas, prostate gland, or skin.

21. The method of claim 17, wherein the indication is Alzheimer's disease, epilepsy, multiple sclerosis, problems associated with tissue grafts and organ transplants, psoriasis, restenosis, stomach ulcers, or tissue overgrowth after surgery, or an infection or infestation 30 of parasites, bacteria, fungi or insects.

22. A method of suppressing cell growth in an individual comprising the step of administering to the individual a therapeutic amount of a polyamine analog of claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15.

5

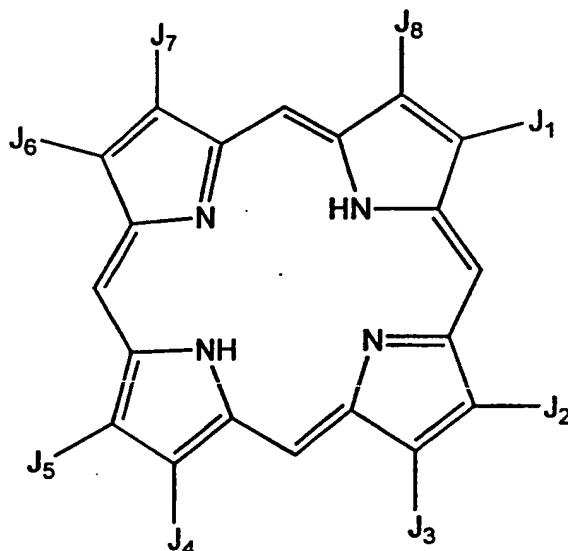
23. The method of claim 22, wherein the individual is a human.

24. A porphyrin-polyamine conjugate, comprising a polyamine compound covalently linked to a porphyrin compound.

10

25. A compound of claim 24, wherein the covalent link is an amide bond or a amine bond.

26. A compound according to claim 24 of the formula



15

wherein at least one of J₁-J₈ is independently selected from the group consisting of

-K₁-G-L-(N(P)-A)_n-K₂

where K₁ is independently selected from the group consisting of C₁-C₈ alkyl and where the valence to the left of K₁ attaches to the porphyrin ring;

20 G is -O-, -(C=O)-, -C(=O)-O-, -O-(C=O)-, -O-(C=O)-O-, -O-(C=O)-N-, -N-(C=O)-O-, or a nonentity;

- L is C₁-C₈ alkyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloaryl, C₁-C₈ alkoxy, C₁-C₈ alkyl-C₃-C₈ cycloalkyl, C₁-C₈ alkyl-C₃-C₈ cycloaryl, C₁-C₈ alkoxy-C₃-C₈ cycloaryl, C₃-C₈ cycloalkyl-C₃-C₈ cycloaryl, C₃-C₈ cycloalkyl-C₁-C₈ alkyl, C₃-C₈ cycloaryl-C₁-C₈ alkoxy, C₃-C₈ cycloaryl-C₃-C₈ cycloalkyl, or a nonentity;
- 5 each A is independently selected from the group consisting of C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloaryl, C₃-C₈ cycloalkenyl, and C₃-C₈ cycloalkynyl;
- P is selected from the group consisting of H and C₁-C₈ alkyl;
- n is an integer from 2 to 8;
- 10 and K₂ is independently selected from the group consisting of H, C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloaryl, C₃-C₈ cycloalkenyl, C₃-C₈ cycloalkynyl, C₁-C₈ alkanol, C₃-C₈ cycloalkanol, and C₃-C₈ hydroxyaryl;
- where the remainder of the groups J₁-J₈ are each independently selected from the group consisting of H, K₃, or K₄-COOH, where K₃ is independently selected from the group
- 15 consisting of C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloaryl, C₃-C₈ cycloalkenyl, C₃-C₈ cycloalkynyl, C₁-C₈ alkanol, C₃-C₈ cycloalkanol, and C₃-C₈ hydroxyaryl; and K₄ is independently selected from the group consisting of C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₈ cycloalkyl, C₃-C₈ cycloaryl, C₃-C₈ cycloalkenyl, and C₃-C₈ cycloalkynyl;
- 20 and any salt or stereoisomer thereof.

27. A compound according to claim 26, wherein K₁ and each A are independently selected from the group consisting of C₁-C₈ alkyl, K₂ is independently selected from the group consisting of H and C₁-C₈ alkyl, and the remainder of the groups J₁-J₈ are each independently selected from the group consisting of H, K₃, or K₄-COOH, where K₃ and K₄ are independently selected from the group consisting of C₁-C₈ alkyl.

28. A compound according to claim 27, wherein K₁ is -CH₂-CH₂- or -CH₂-CH₂-CH₂-.
- 30 29. A compound according to claim 27, wherein n is 4.

30. A compound according to claim 27, wherein J₃, J₄, J₇ and J₈ are independently selected from the group consisting of C₁-C₃ alkyl;

J₅ and J₆ are independently selected from the group consisting of C₁-C₃ alkyl and C₁-C₃ alkyl-COOH;

5 and J₁ and J₂ are independently selected from the group consisting of

C₁-C₃ alkyl-G-N(P₁)-A-(NH-A)_{n1}-K₅;

wherein G is -(C=O)- or a nonentity;

P₁ is H or C₁-C₃ alkyl;

each A is independently selected from the group consisting of C₁-C₈ alkyl;

10 n₁ is 3 or 4;

and K₅ is independently selected from the group consisting of H and C₁-C₈ alkyl.

31. A method of treating an indication in an individual comprising the step of administering to the individual a therapeutic amount of a porphyrin-polyamine conjugate of

15 claim 24.

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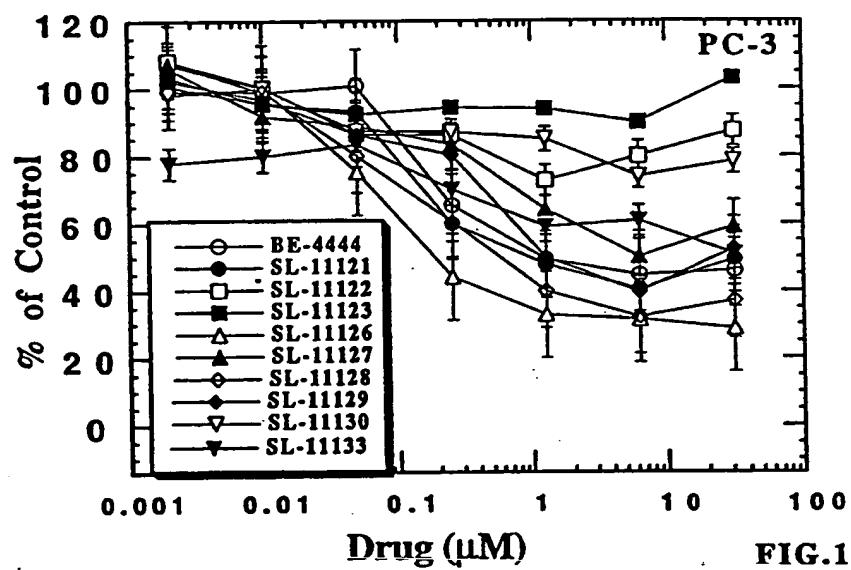


FIG.1

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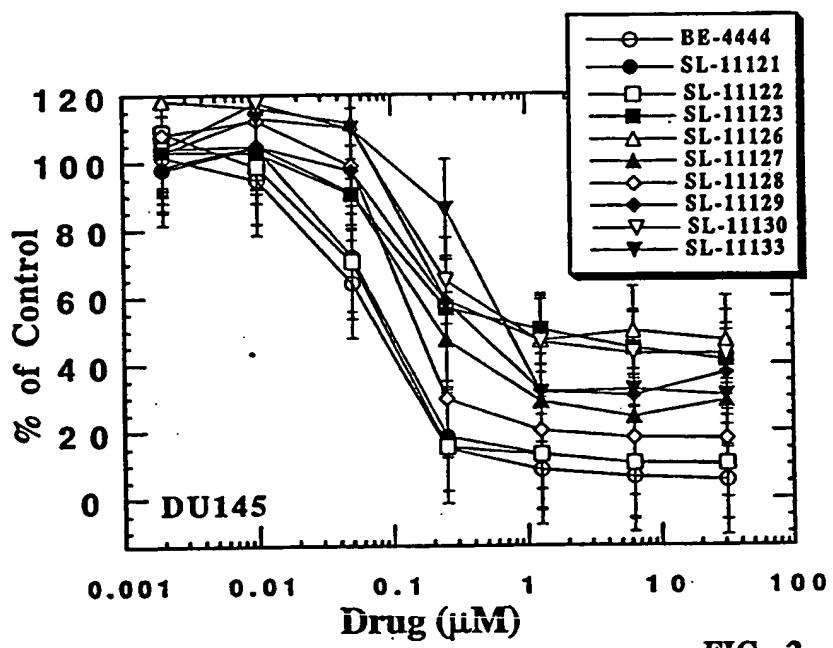


FIG. 2

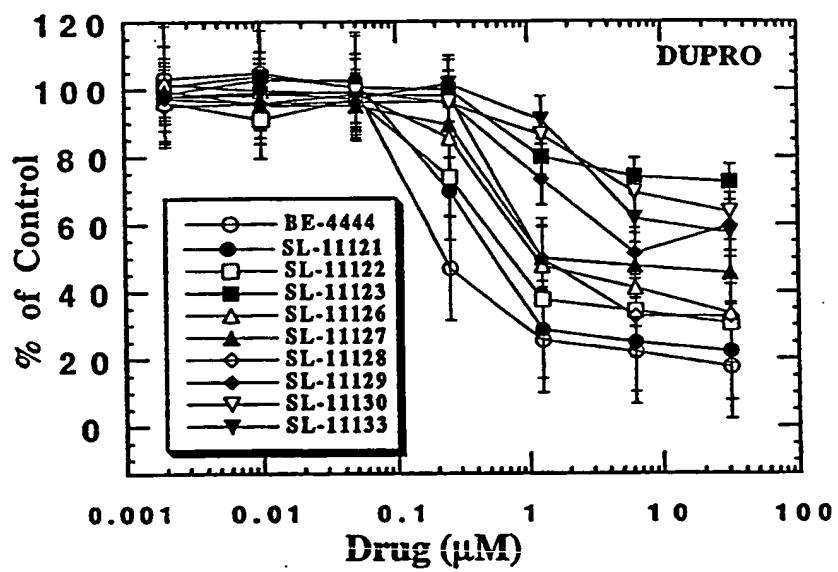


FIG. 3

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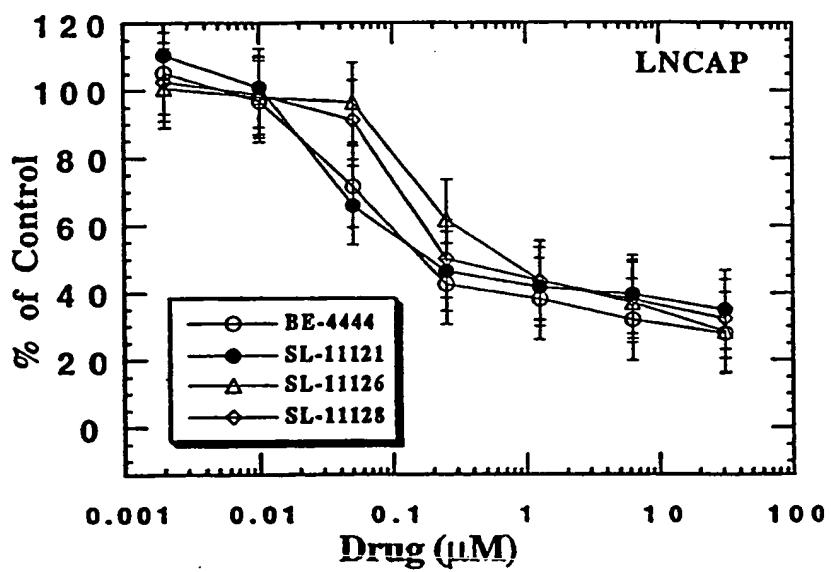


FIG. 4

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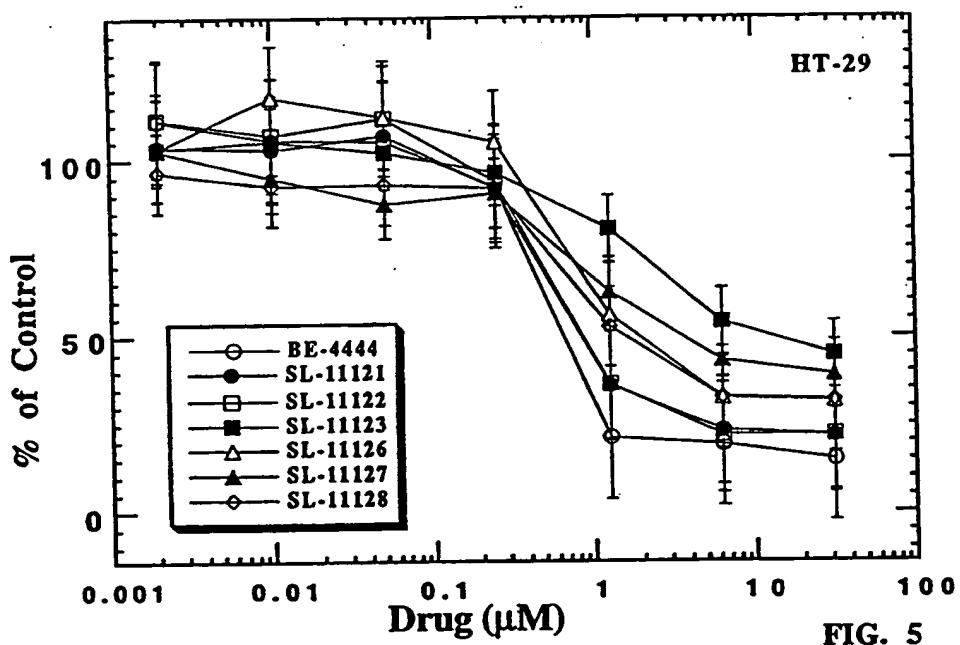


FIG. 5

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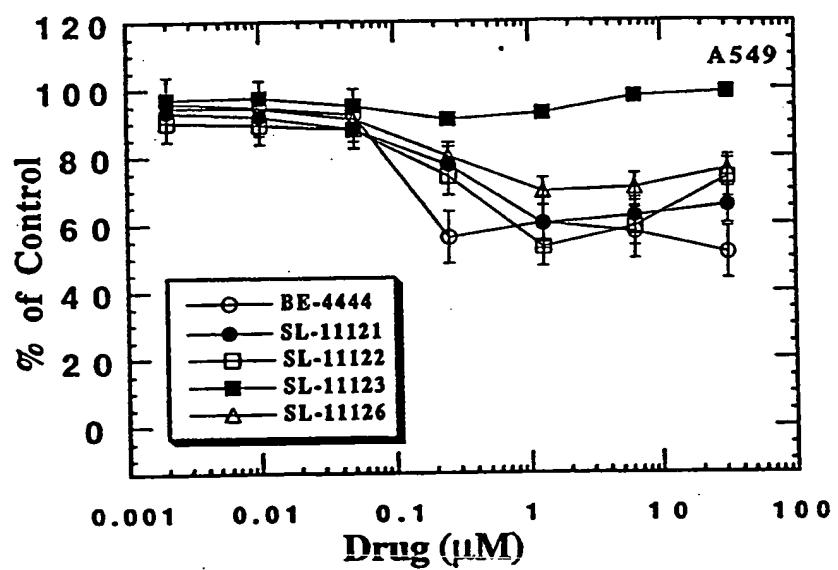


FIG. 6

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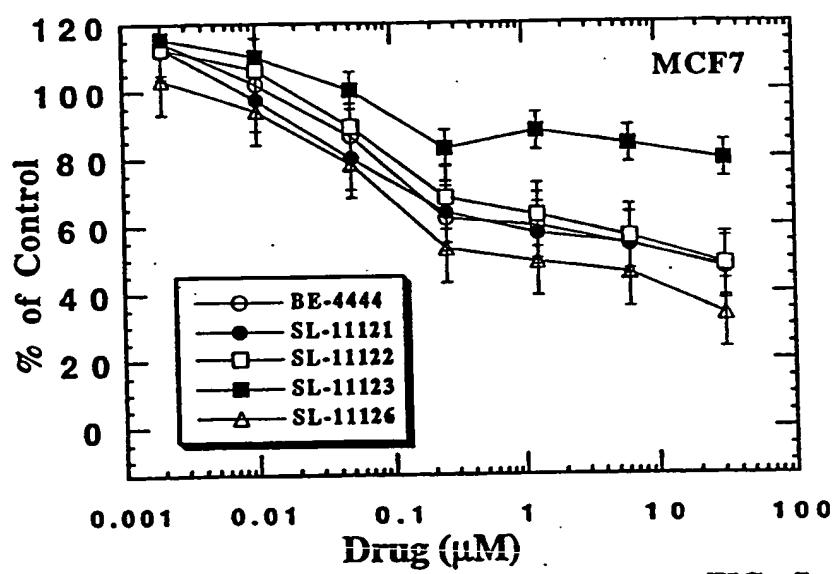


FIG. 7

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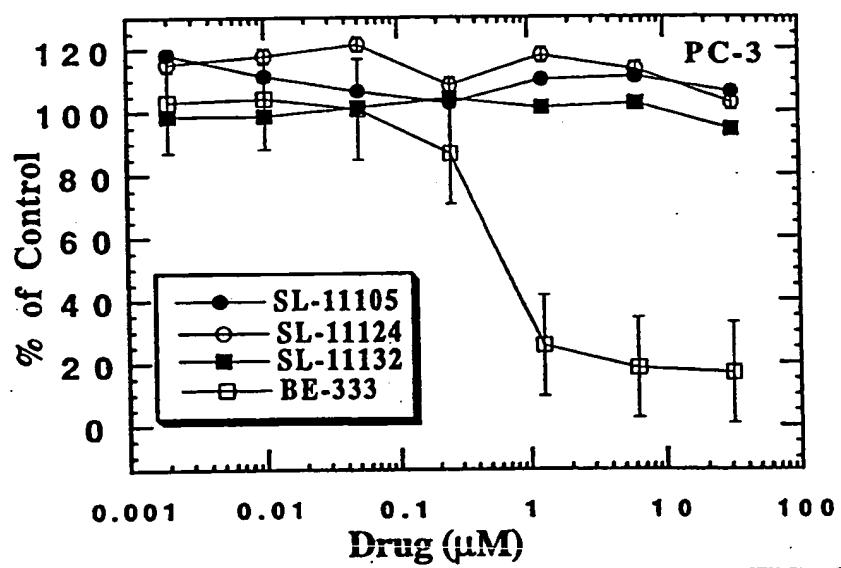


FIG. 8

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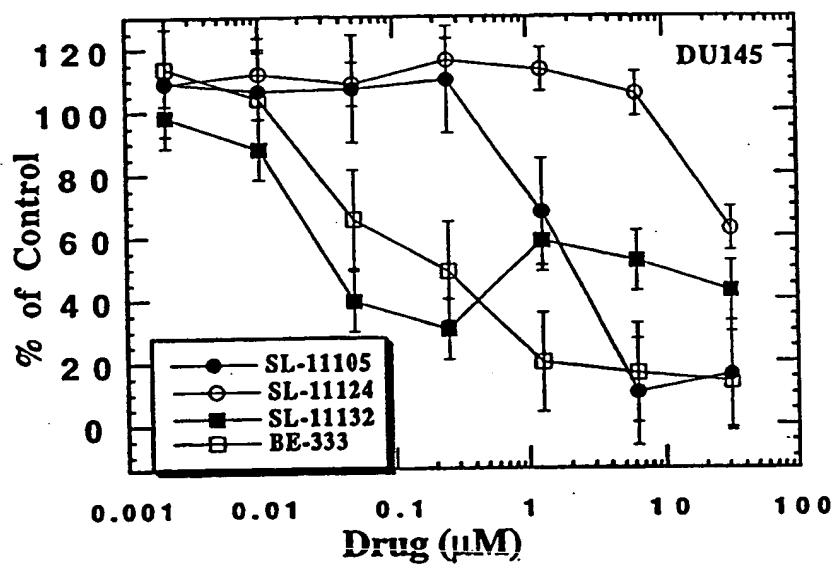


FIG. 9

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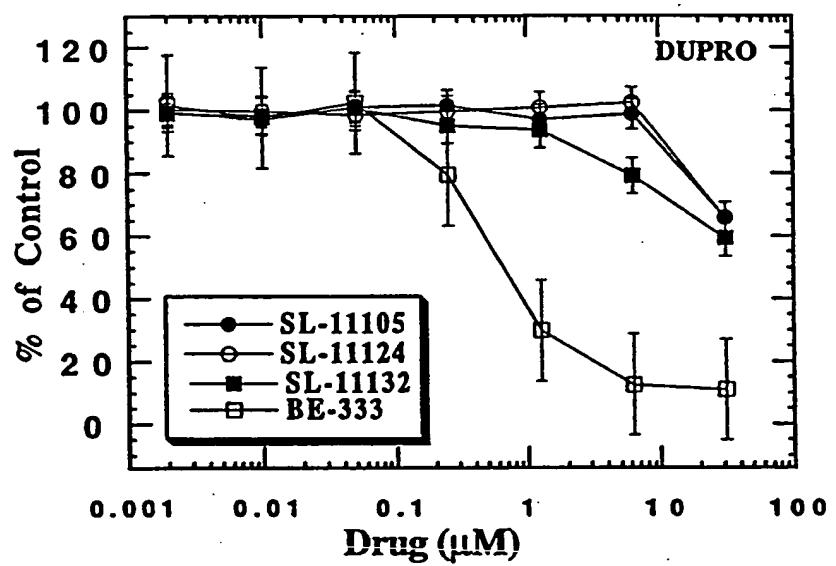


FIG. 10

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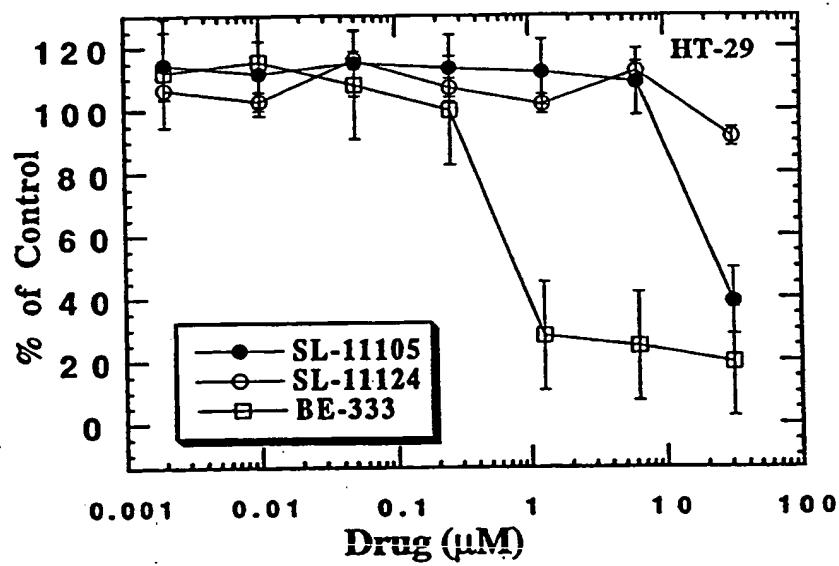


FIG. 11

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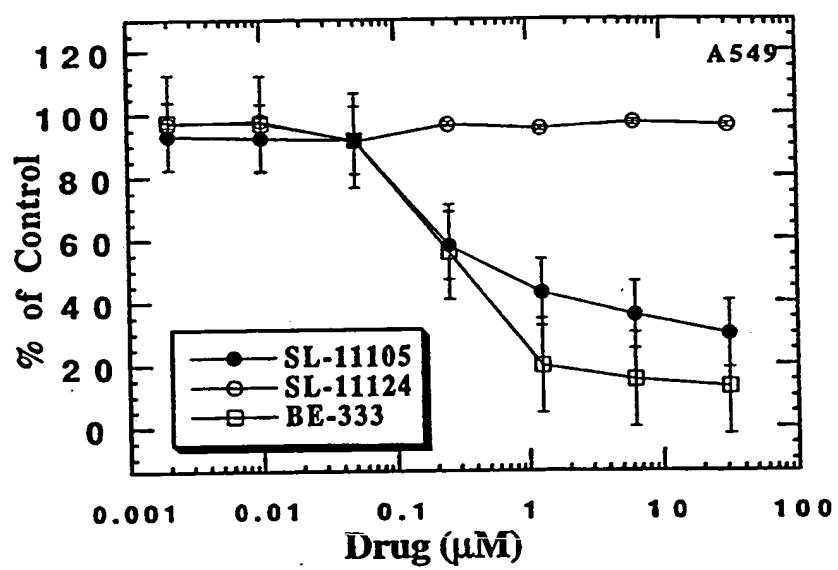


FIG. 12

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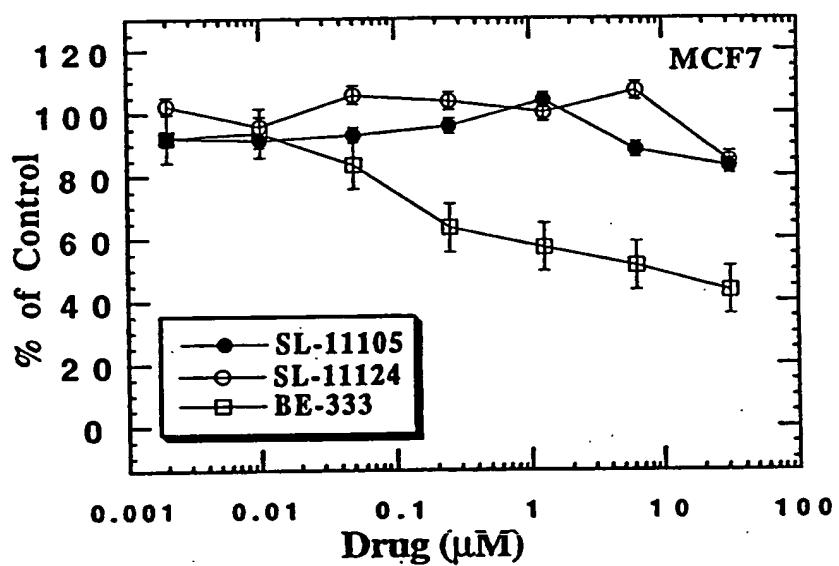


FIG. 13

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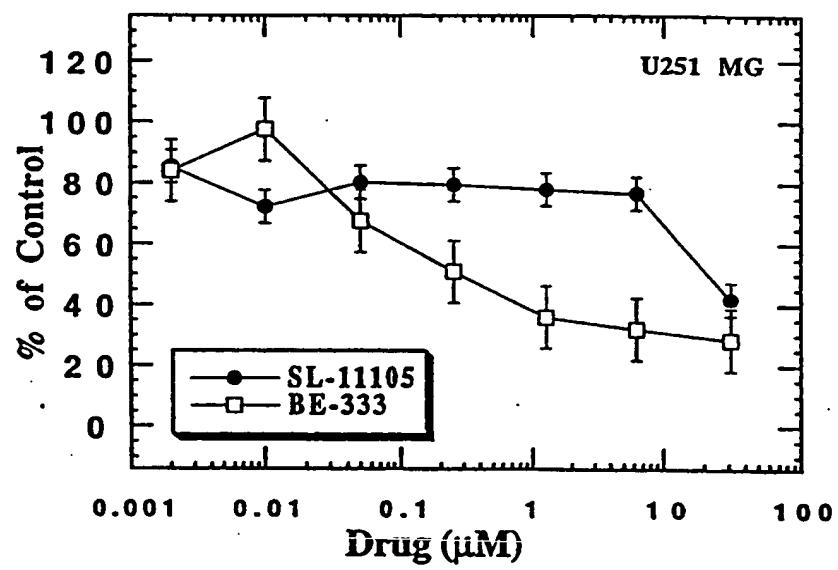


FIG. 14

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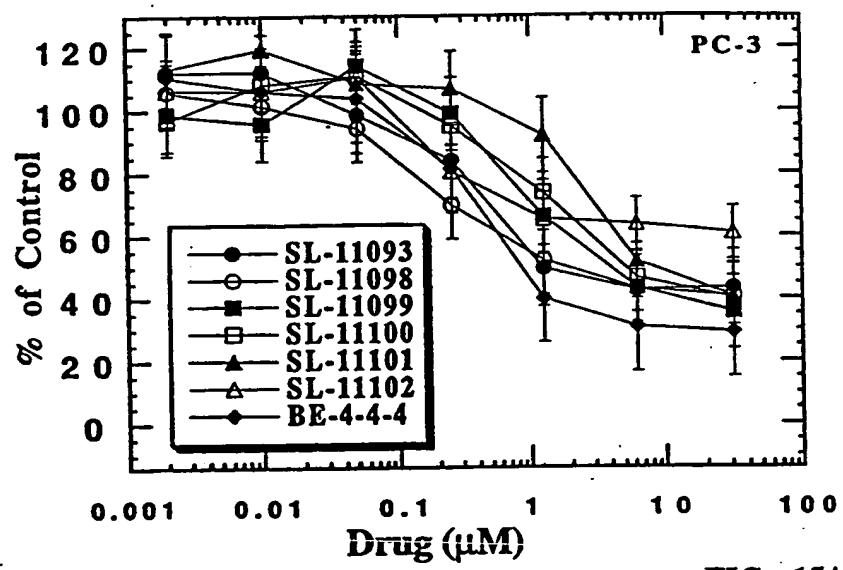


FIG. 15A

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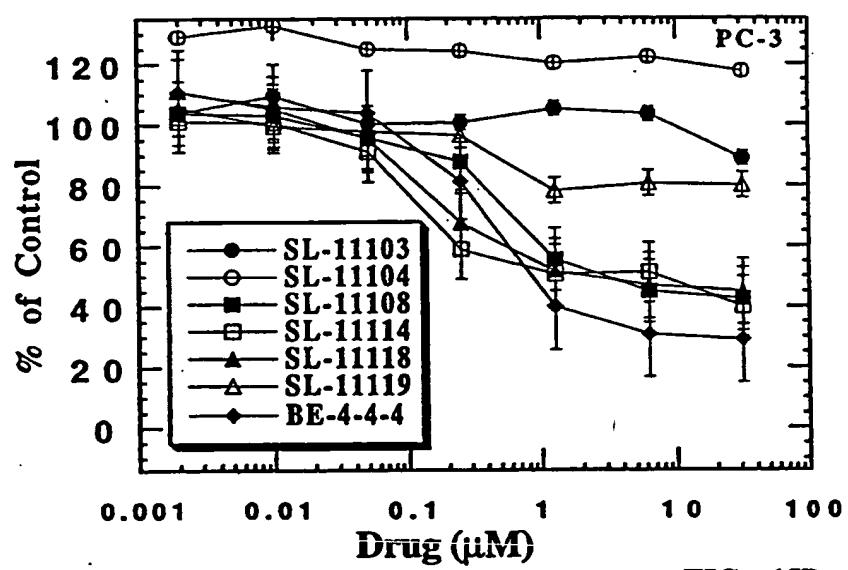


FIG. 15B

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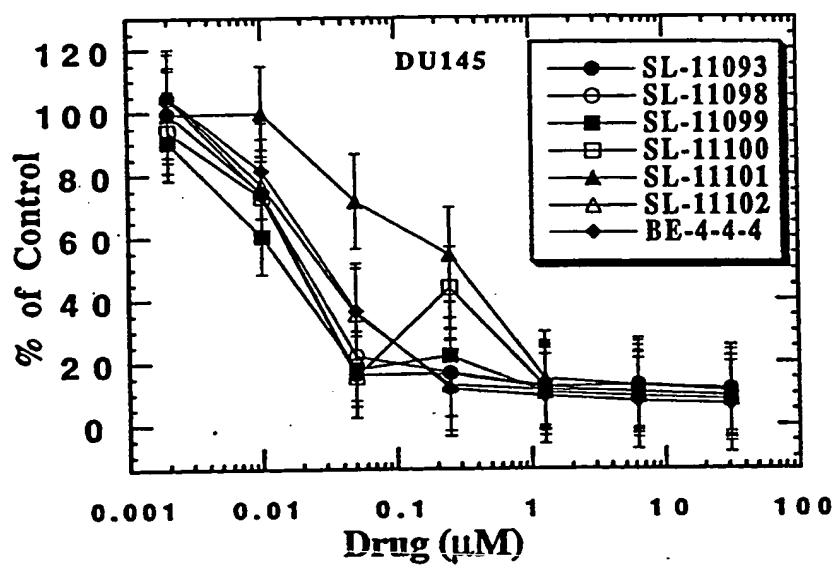


FIG. 16 A

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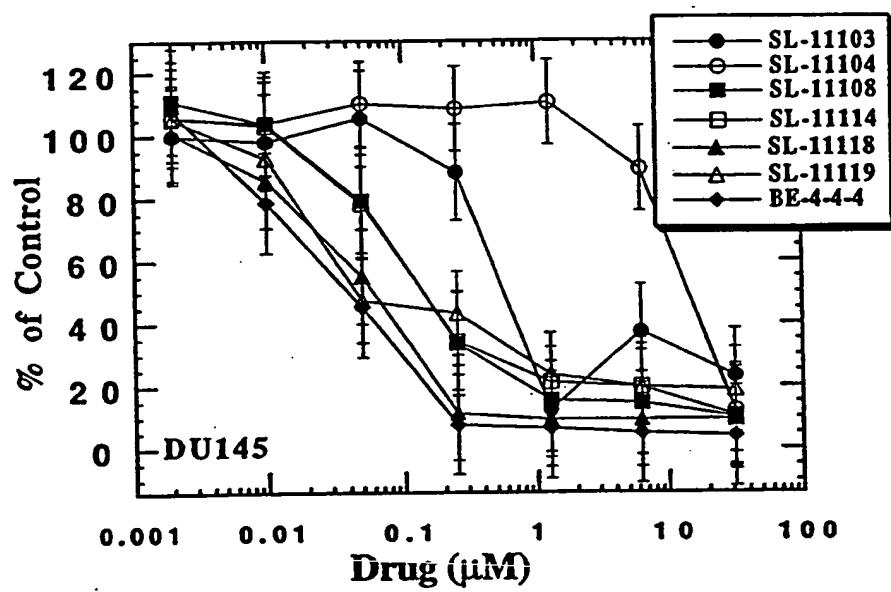


FIG. 16 B

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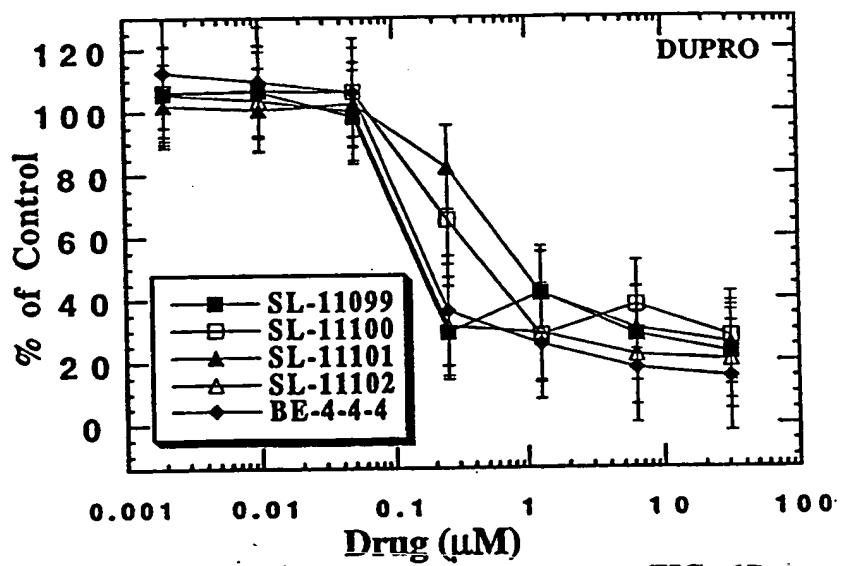


FIG. 17 A

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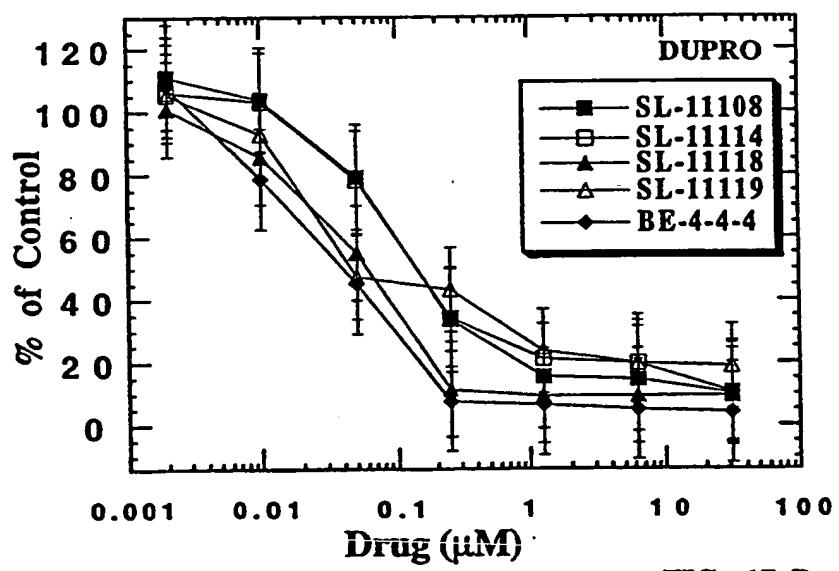


FIG. 17 B

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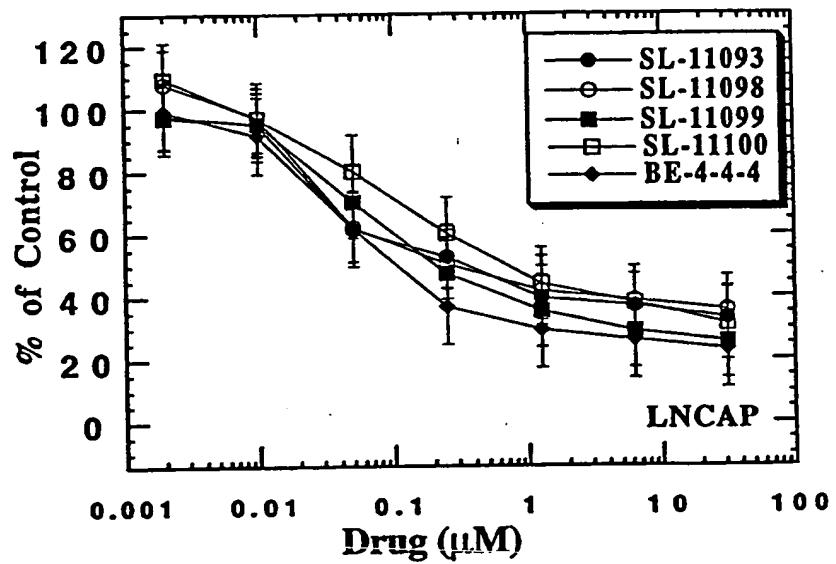


FIG. 18 A

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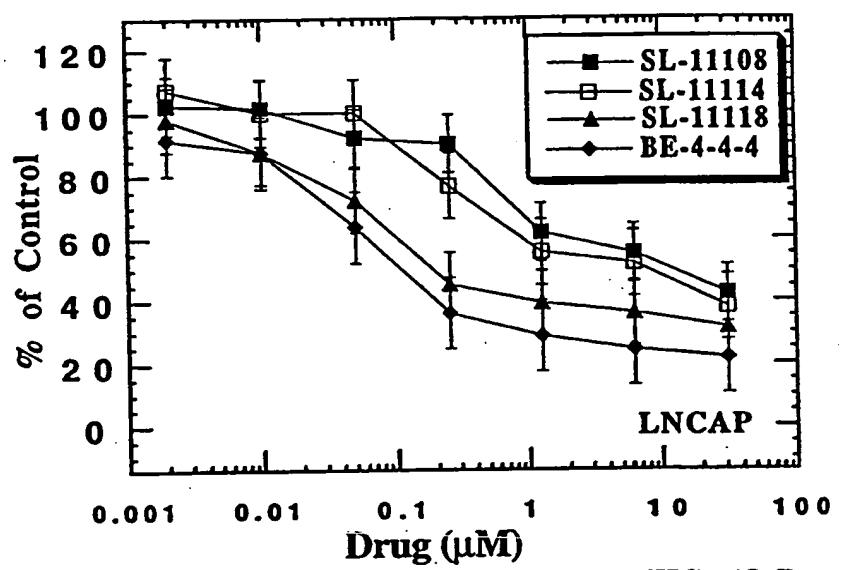


FIG. 18 B

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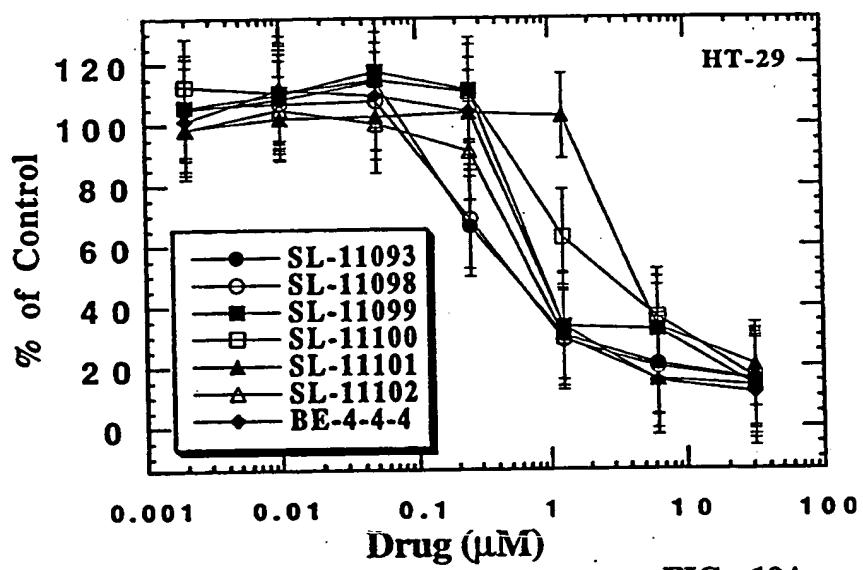


FIG. 19A

24156

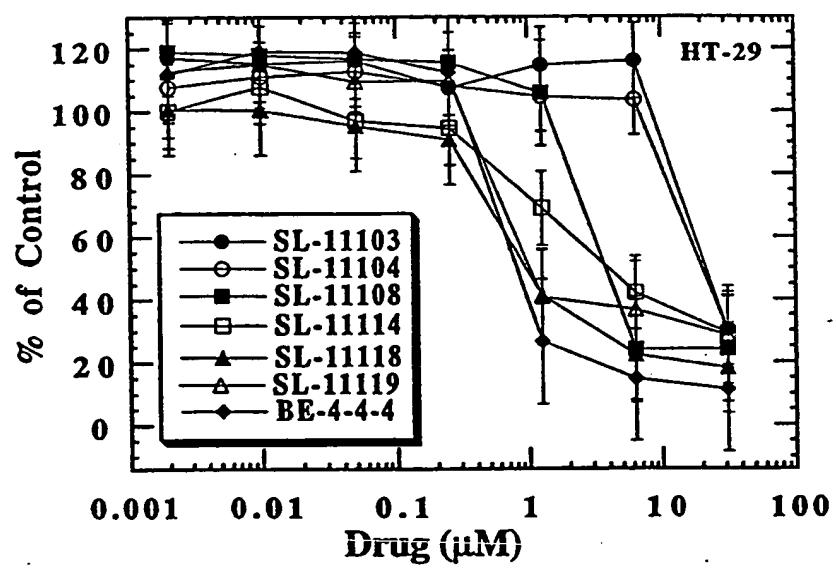


FIG. 19B

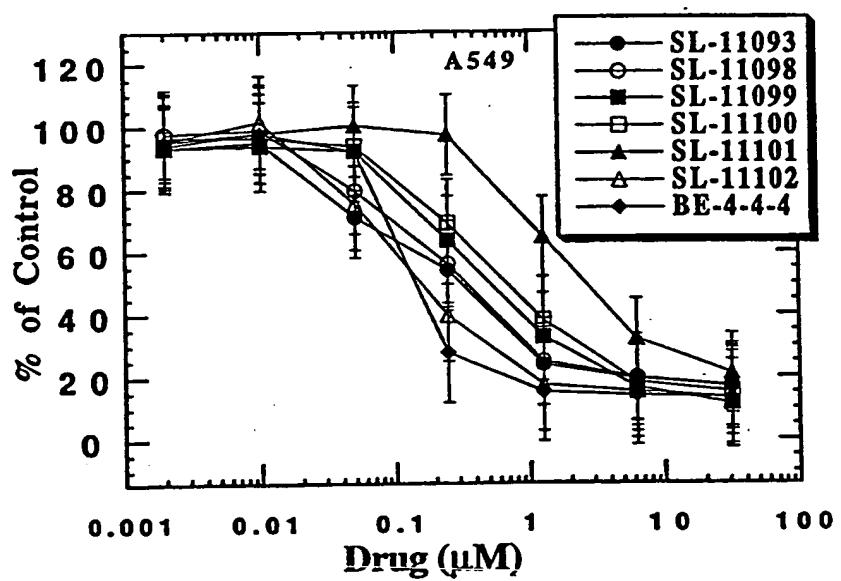


FIG. 20 A

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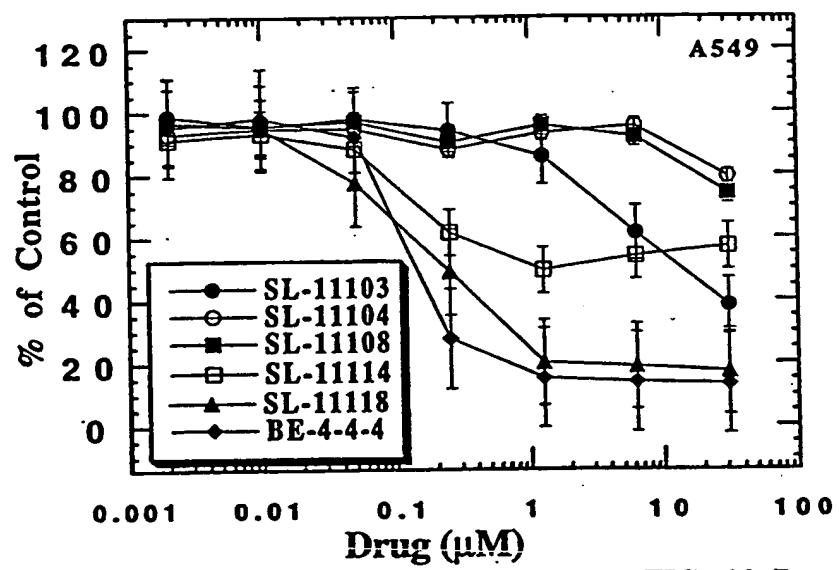


FIG. 20 B

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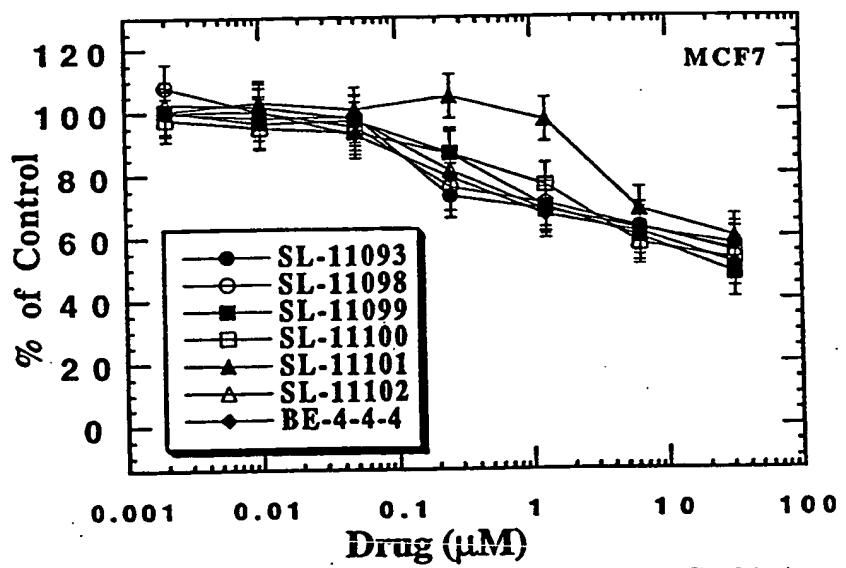


FIG. 21 A

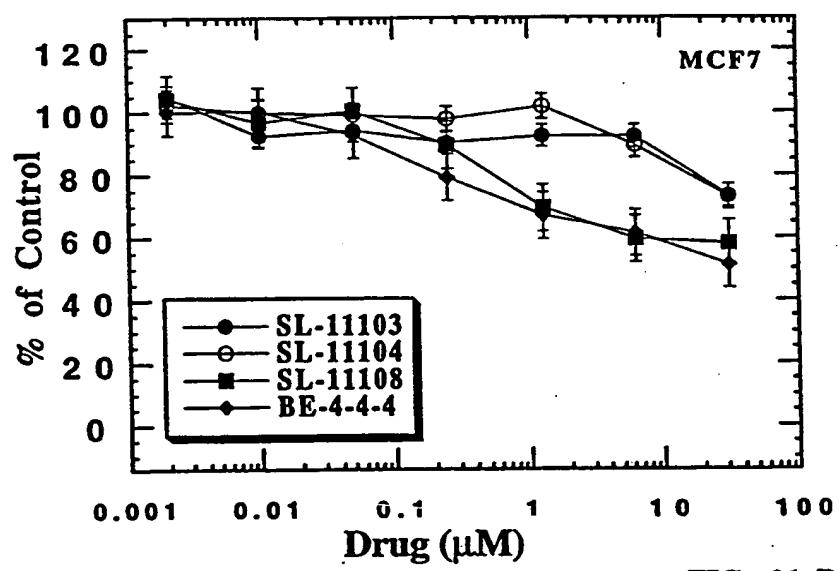


FIG. 21 B

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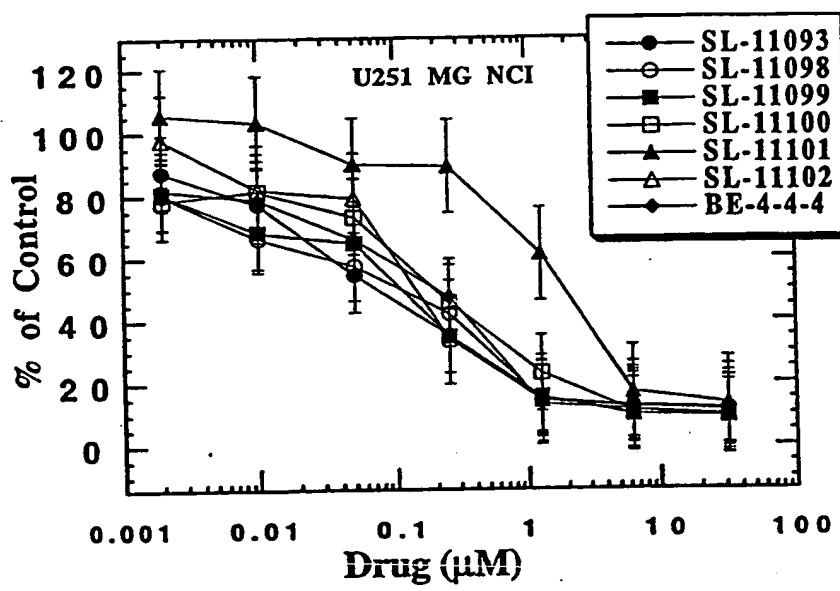


FIG. 22 A

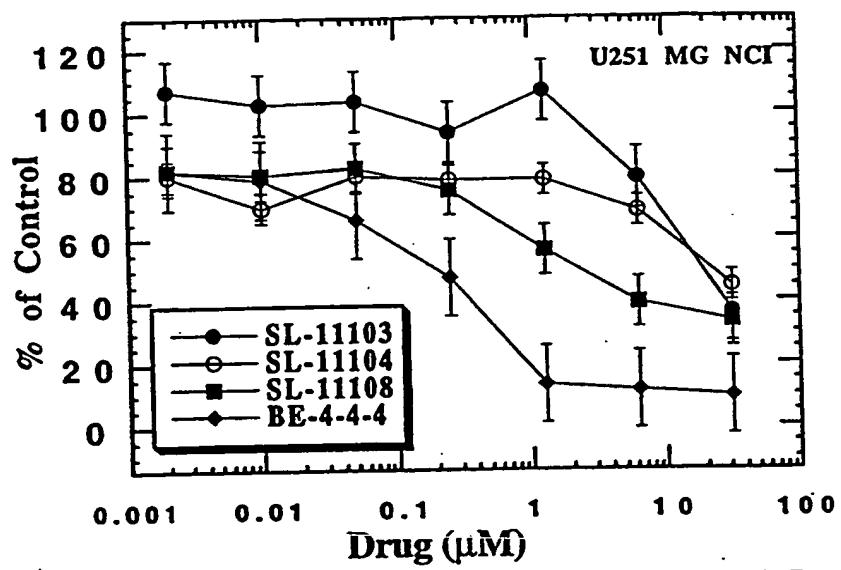


FIG. 22 B

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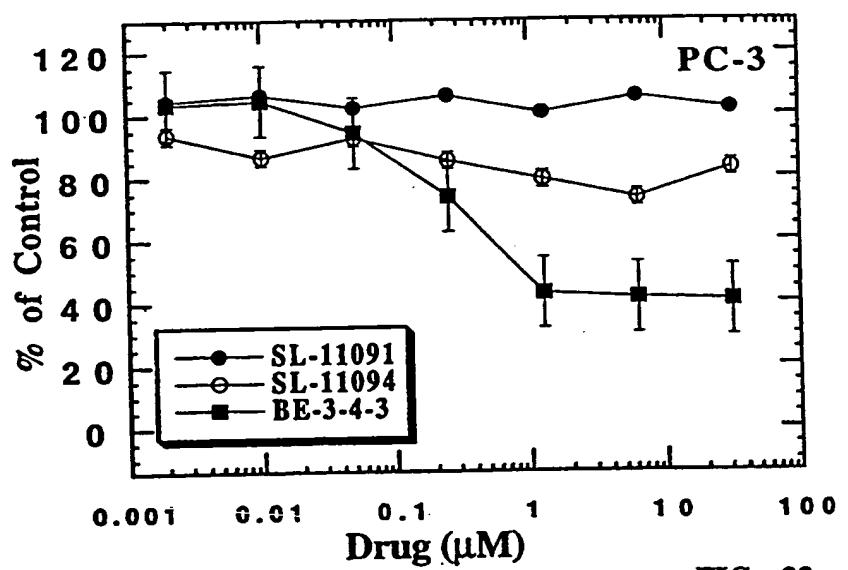


FIG. 23

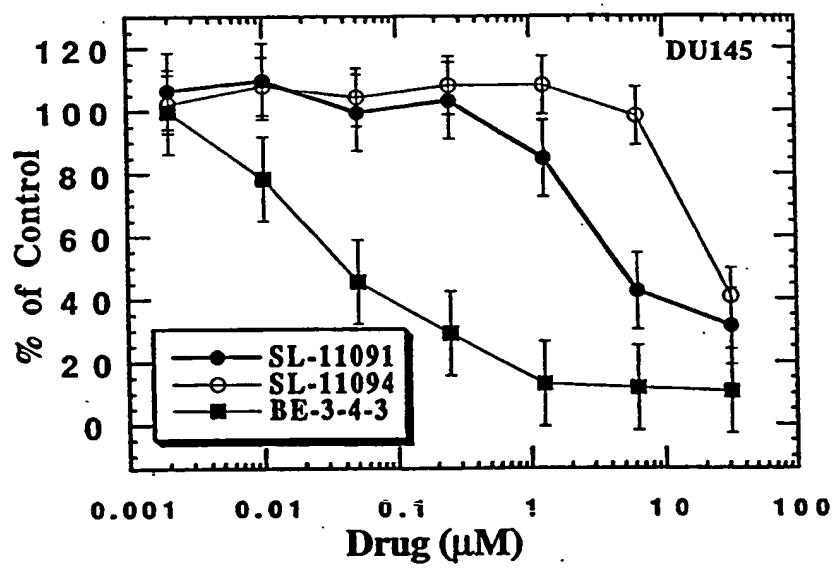


FIG. 24

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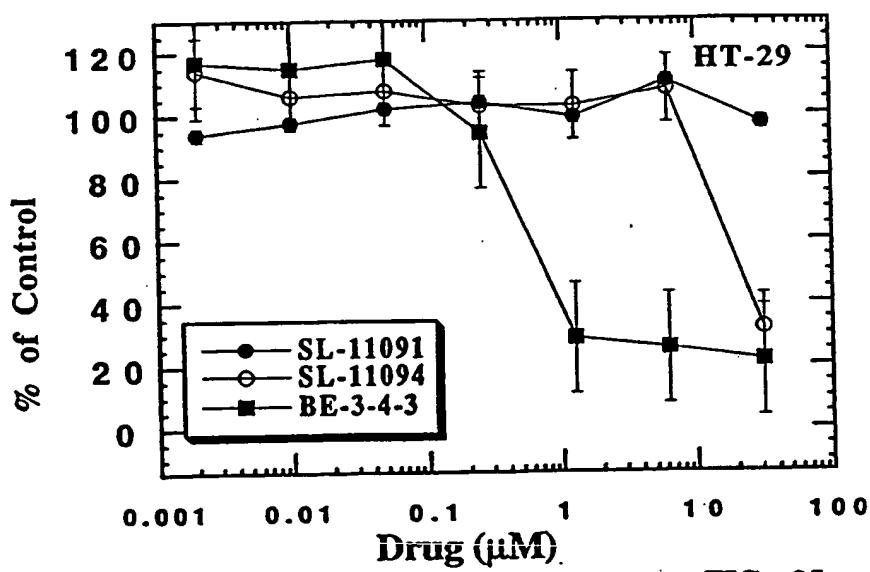


FIG. 25

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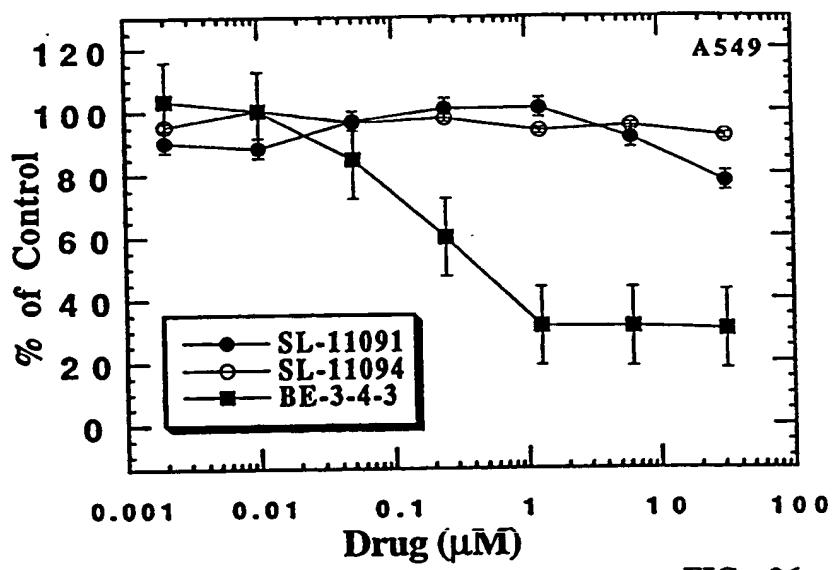


FIG. 26

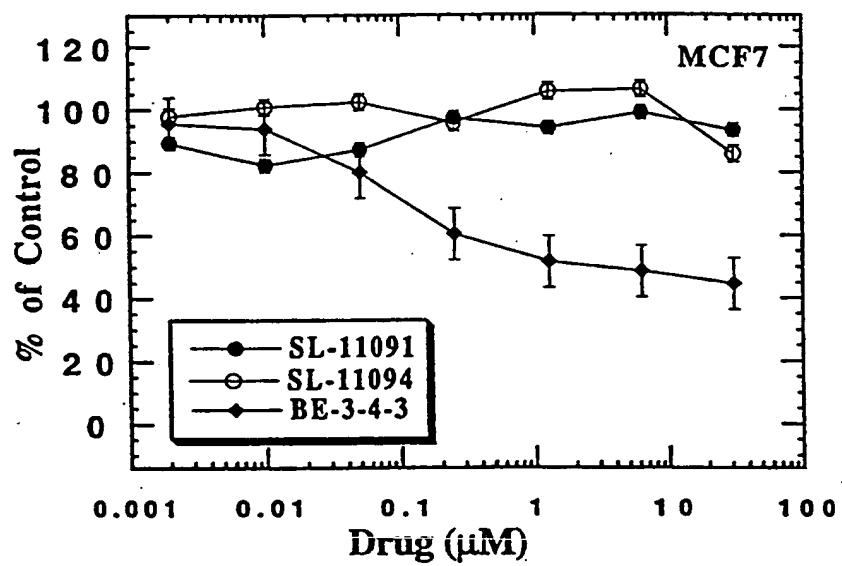


FIG. 27

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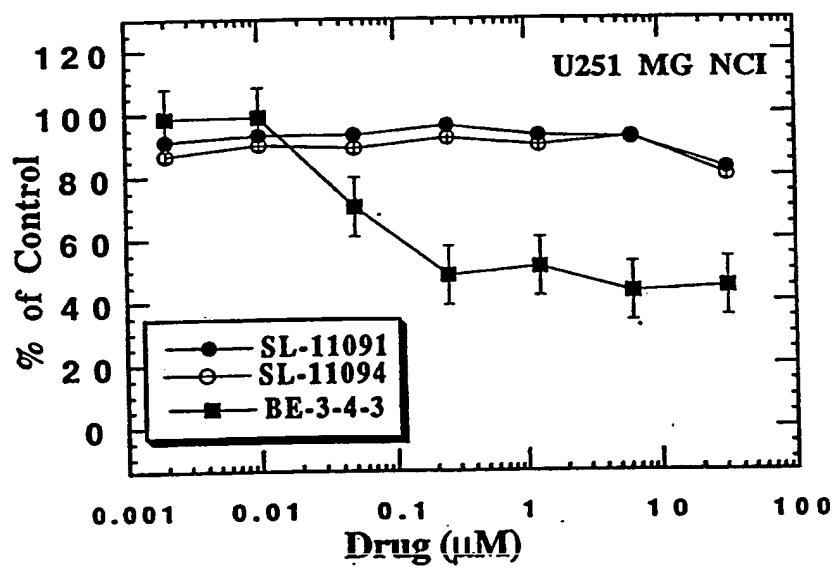
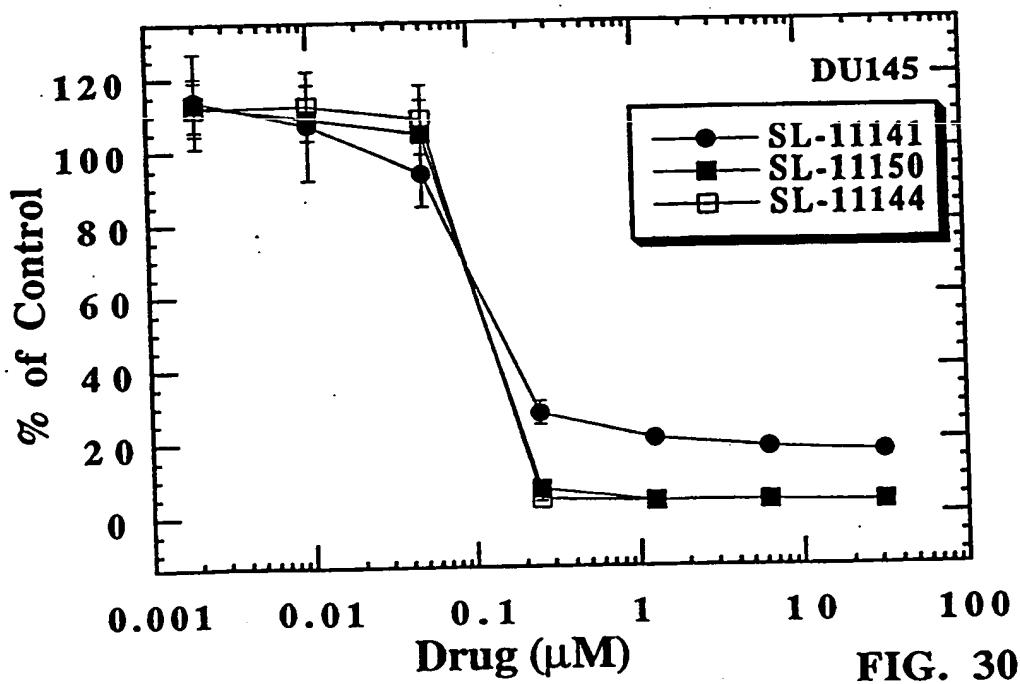
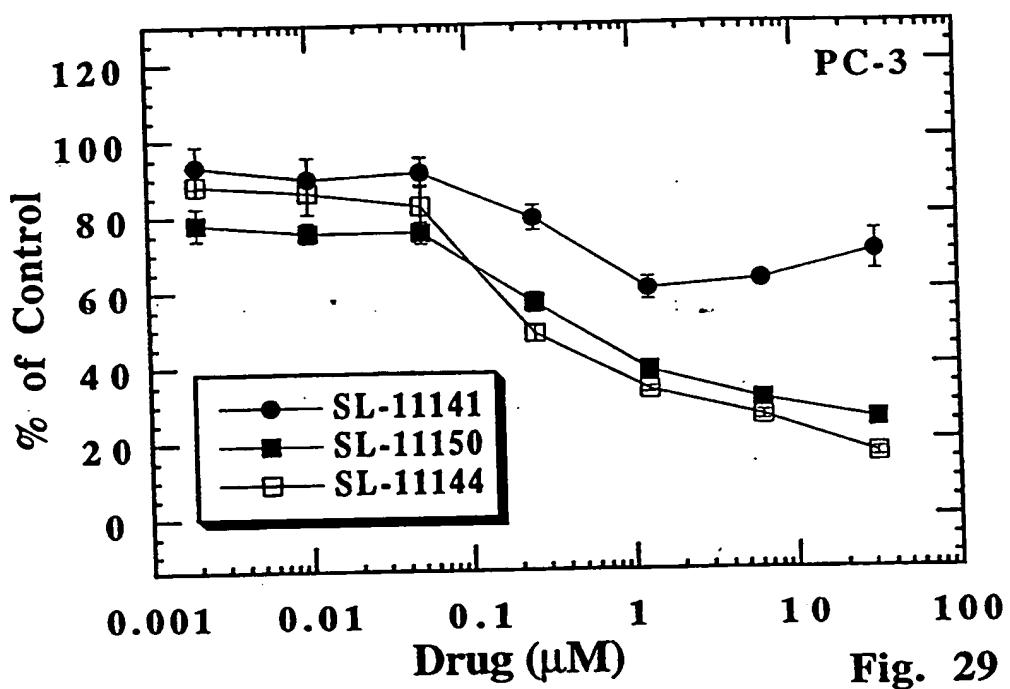
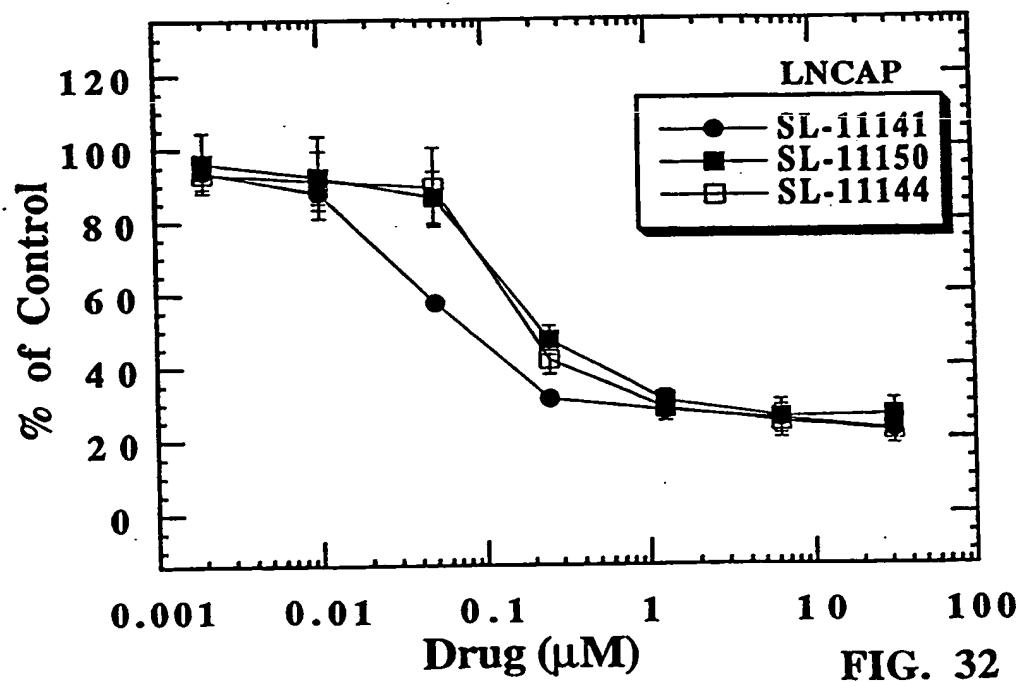
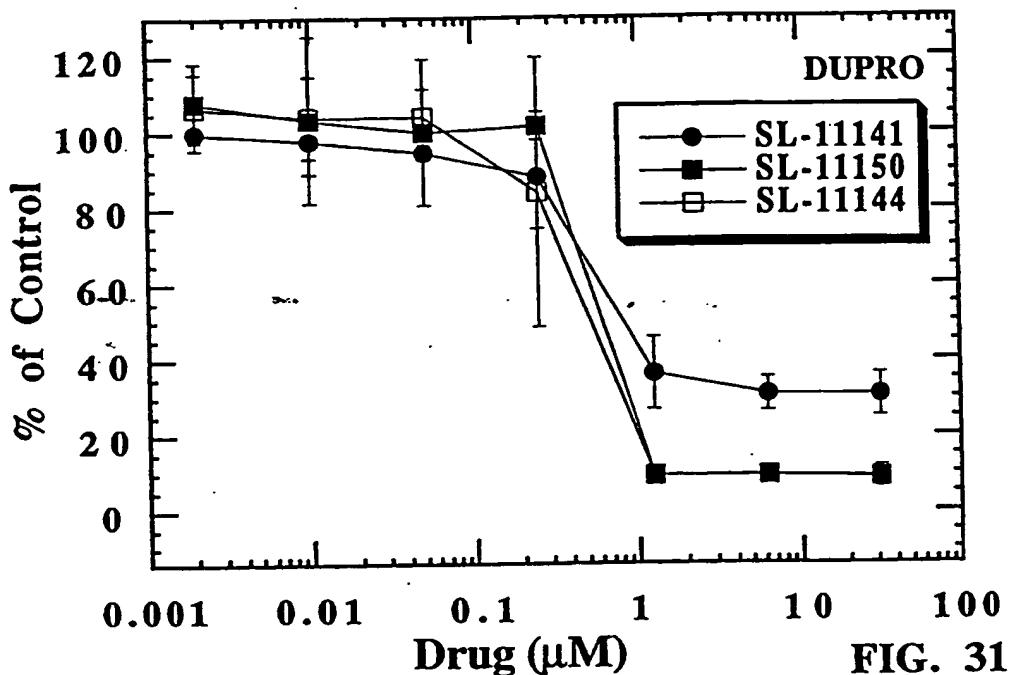


FIG. 28

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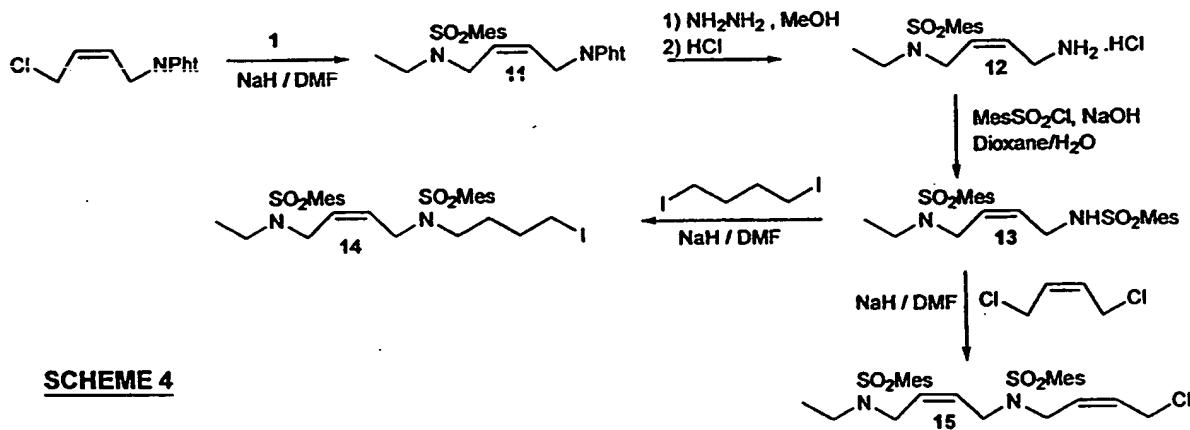
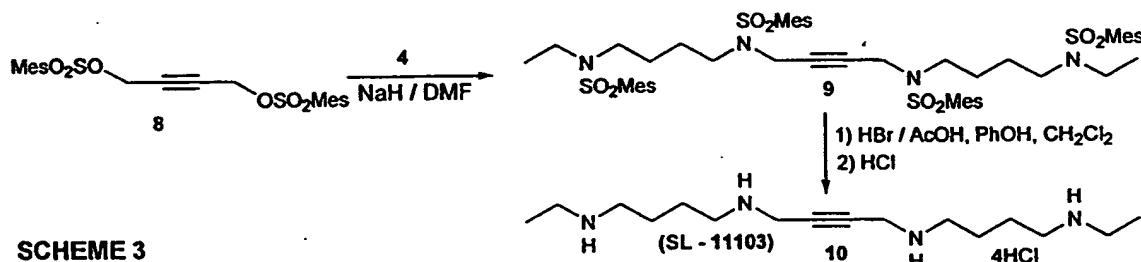
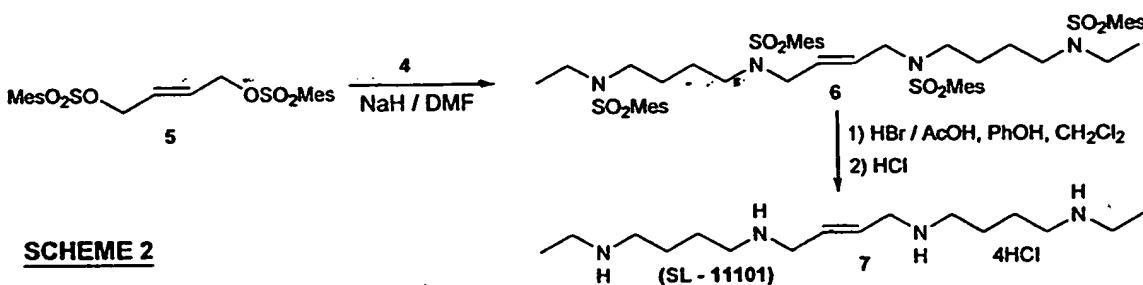
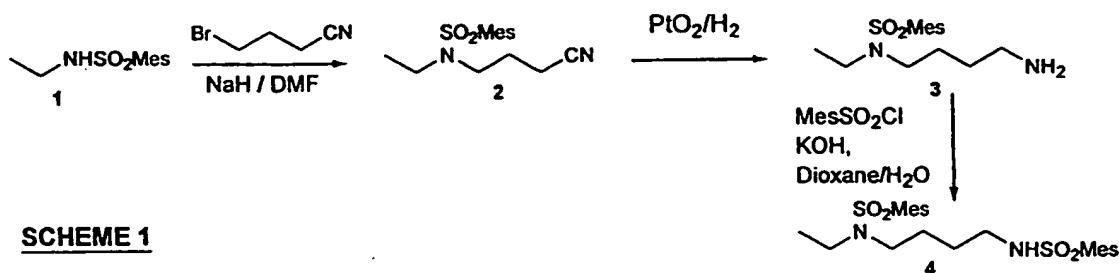


Figure 34

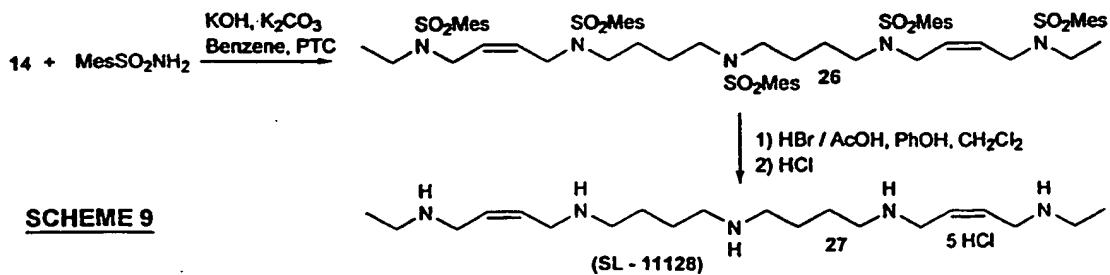
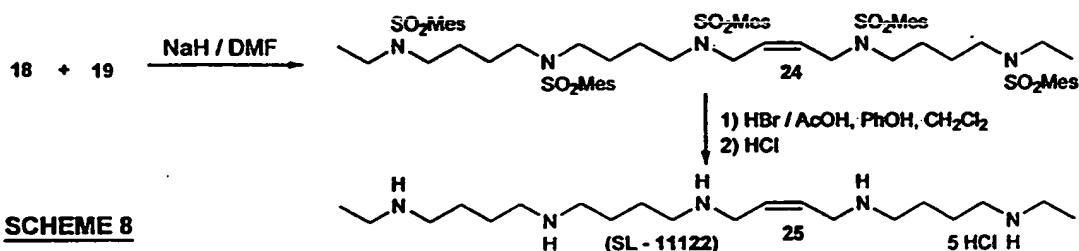
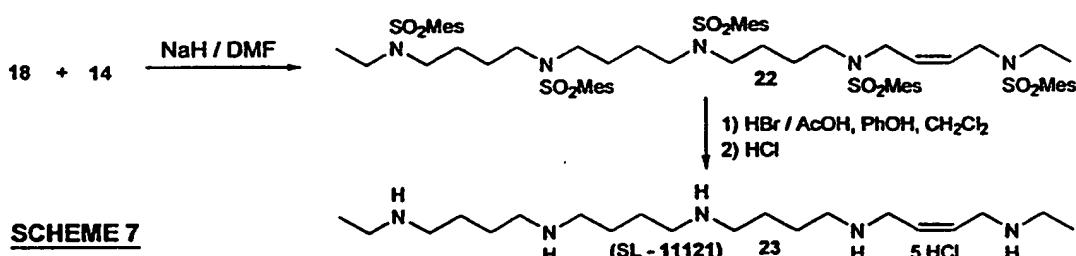
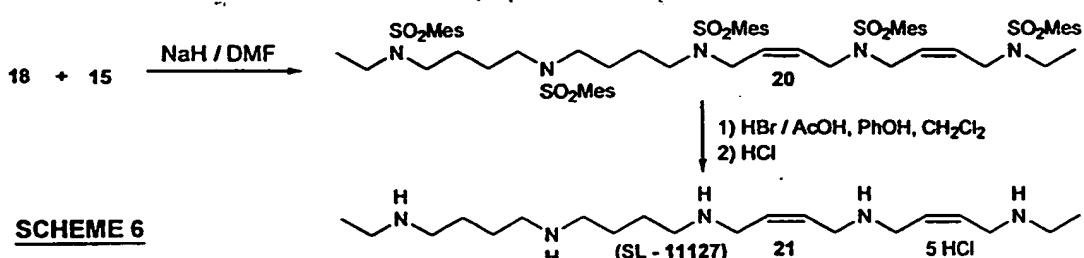
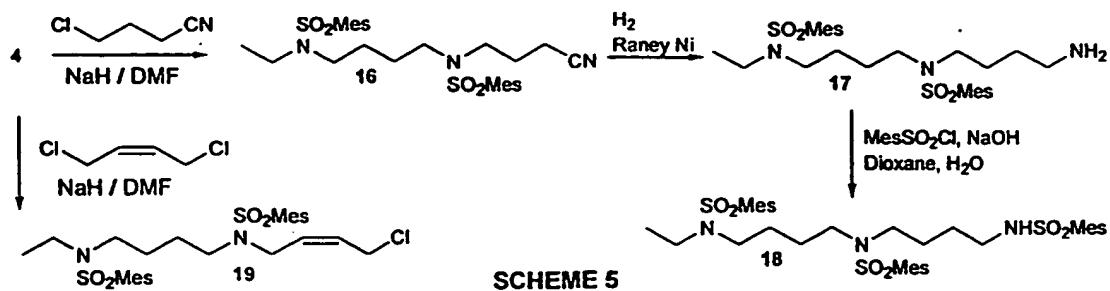
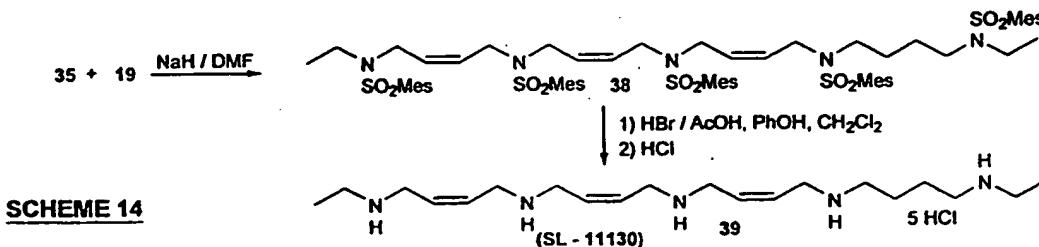
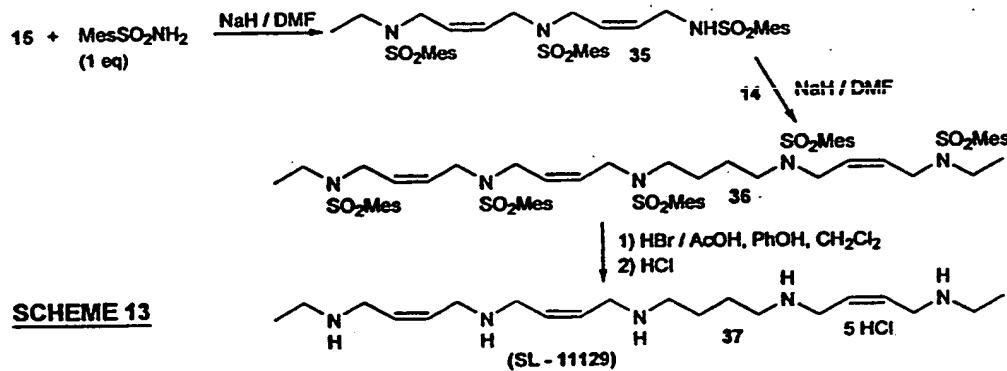
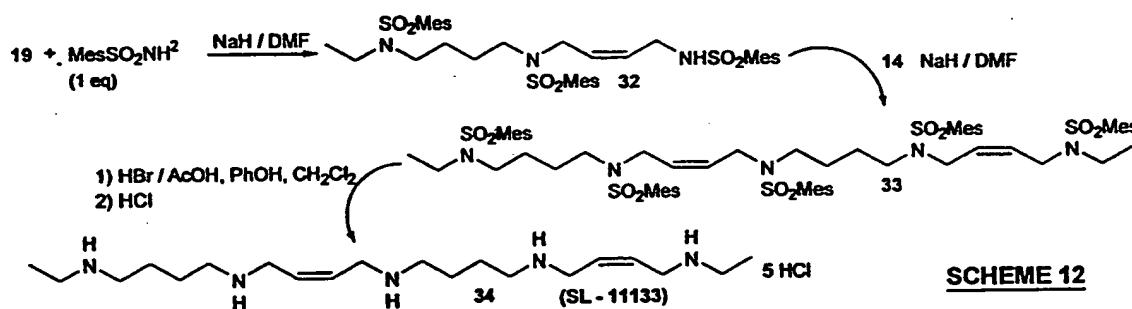
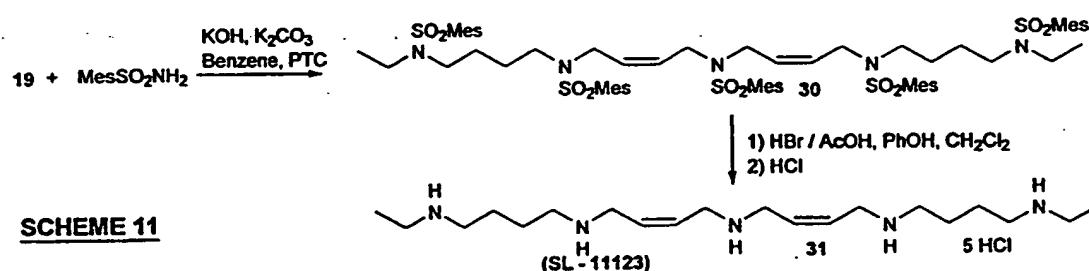
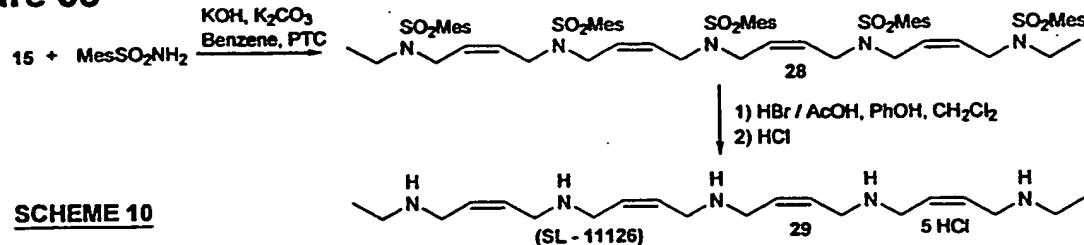


Figure 35



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Figure 36

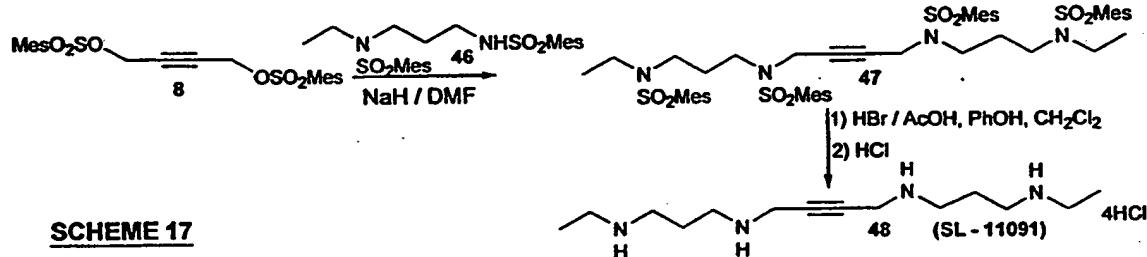
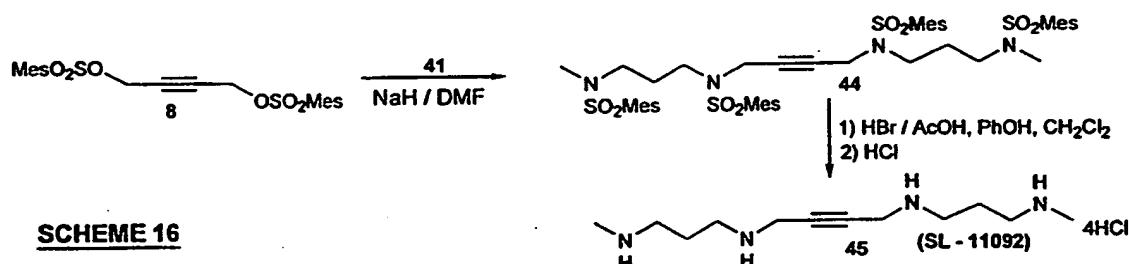
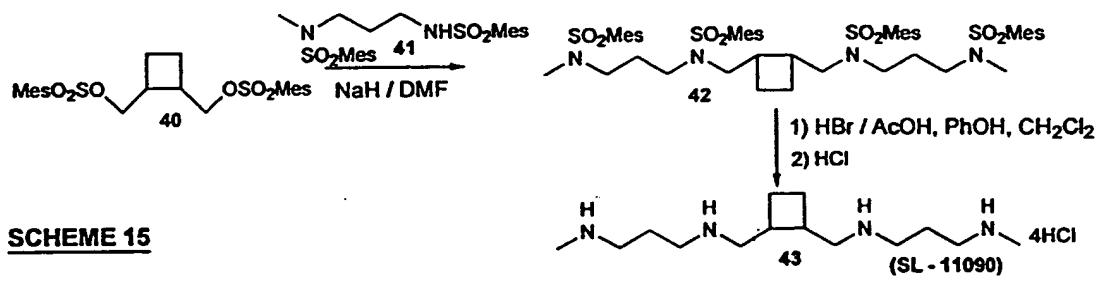
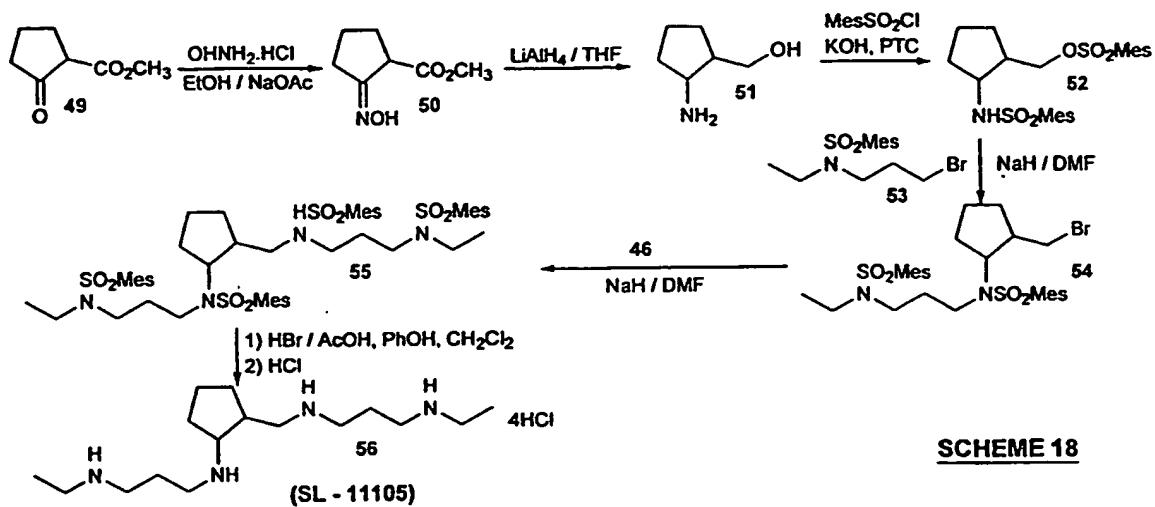
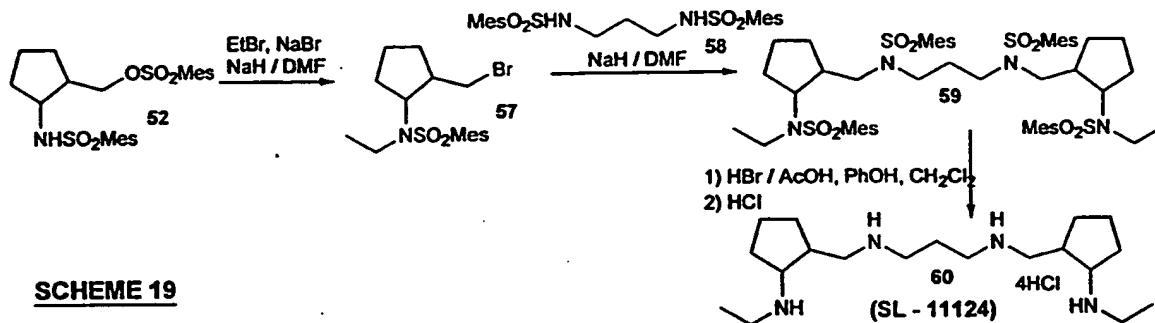


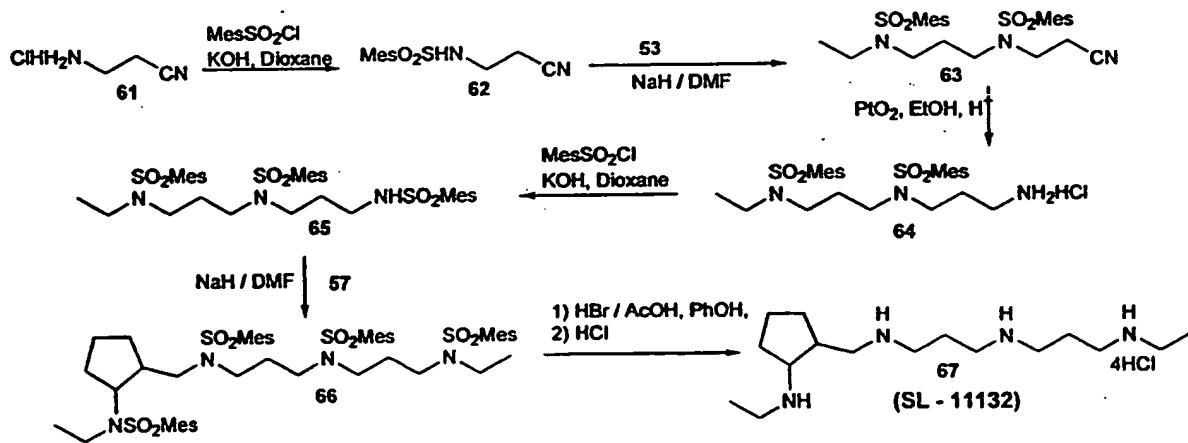
Figure 37



SCHEME 18



SCHEME 19



SCHEME 20

Figure 38

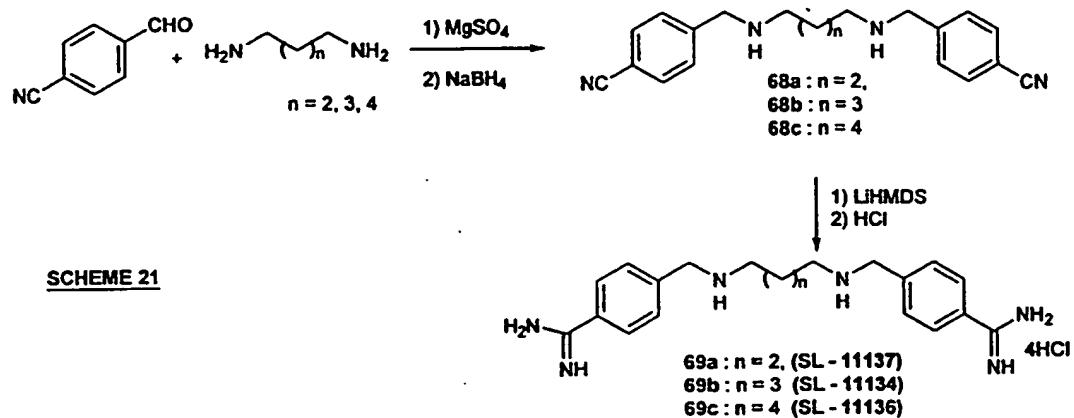
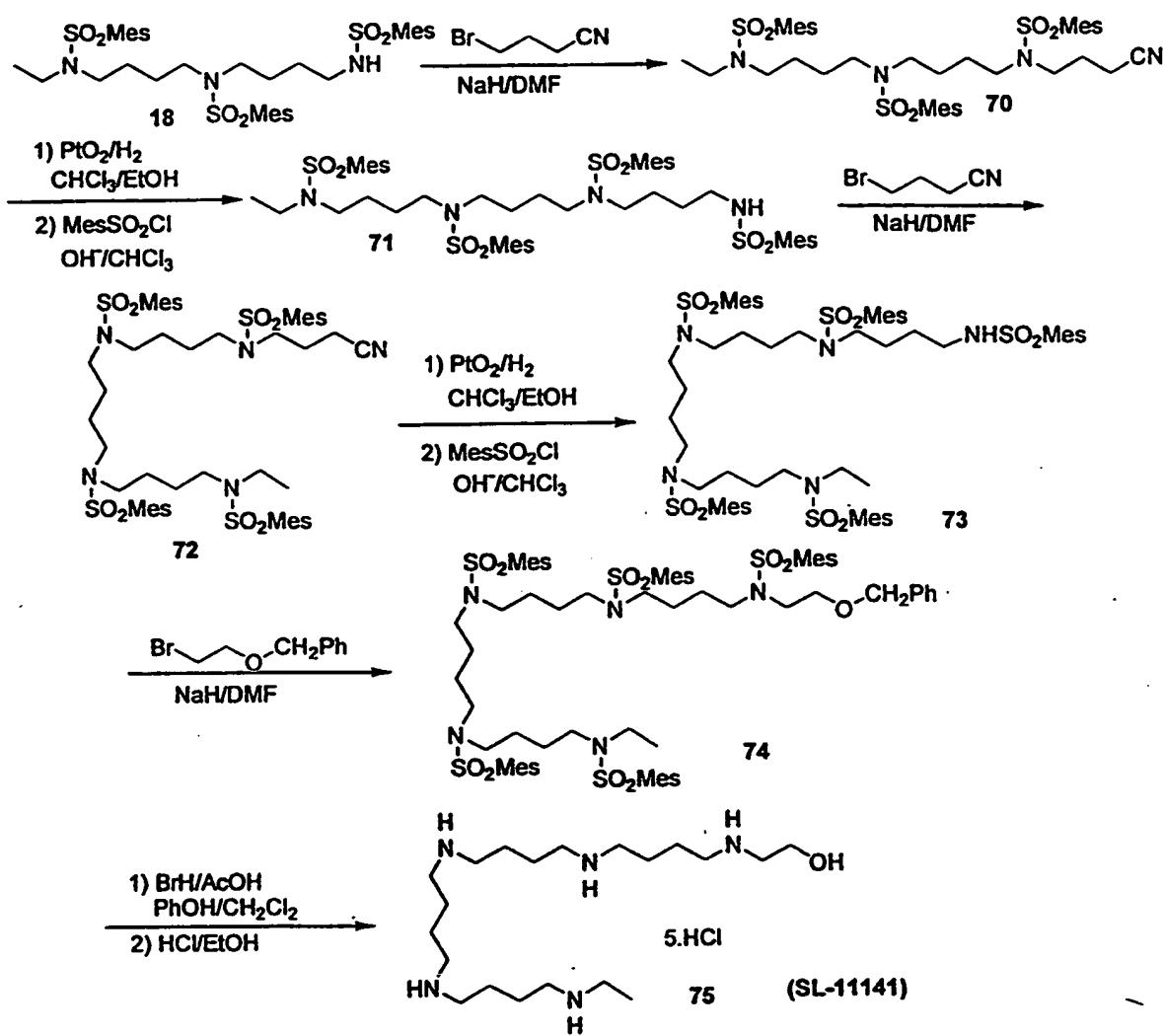
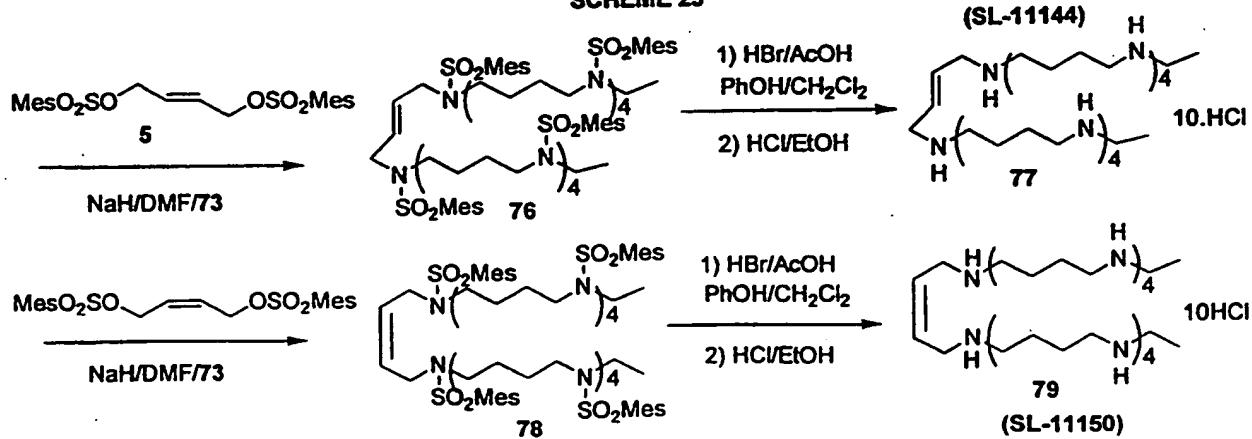


Figure 39



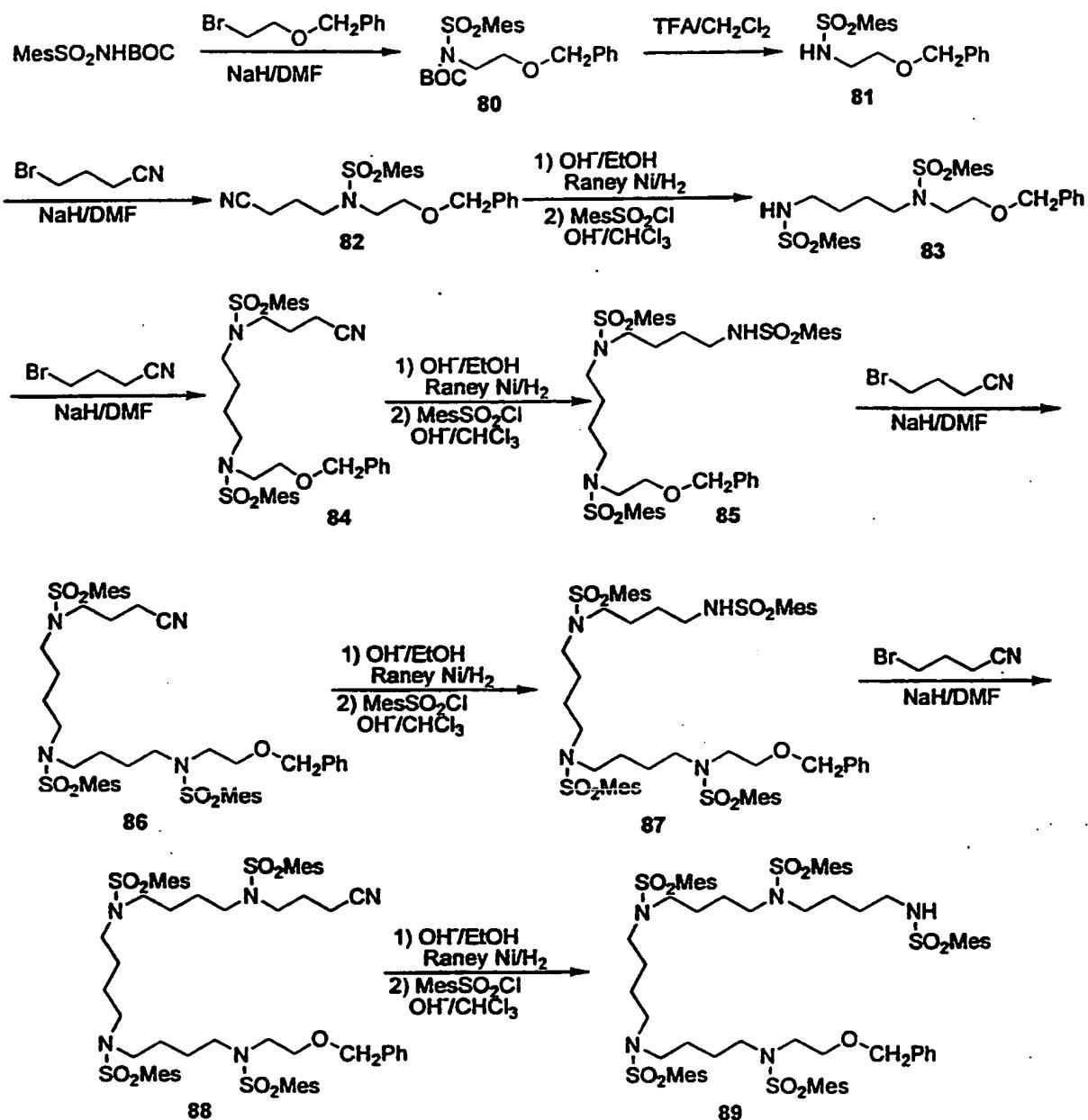
SCHEME 23



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Figure 40A

SCHEME 24



47156

Figure 40B

SCHEME 24 (continued)

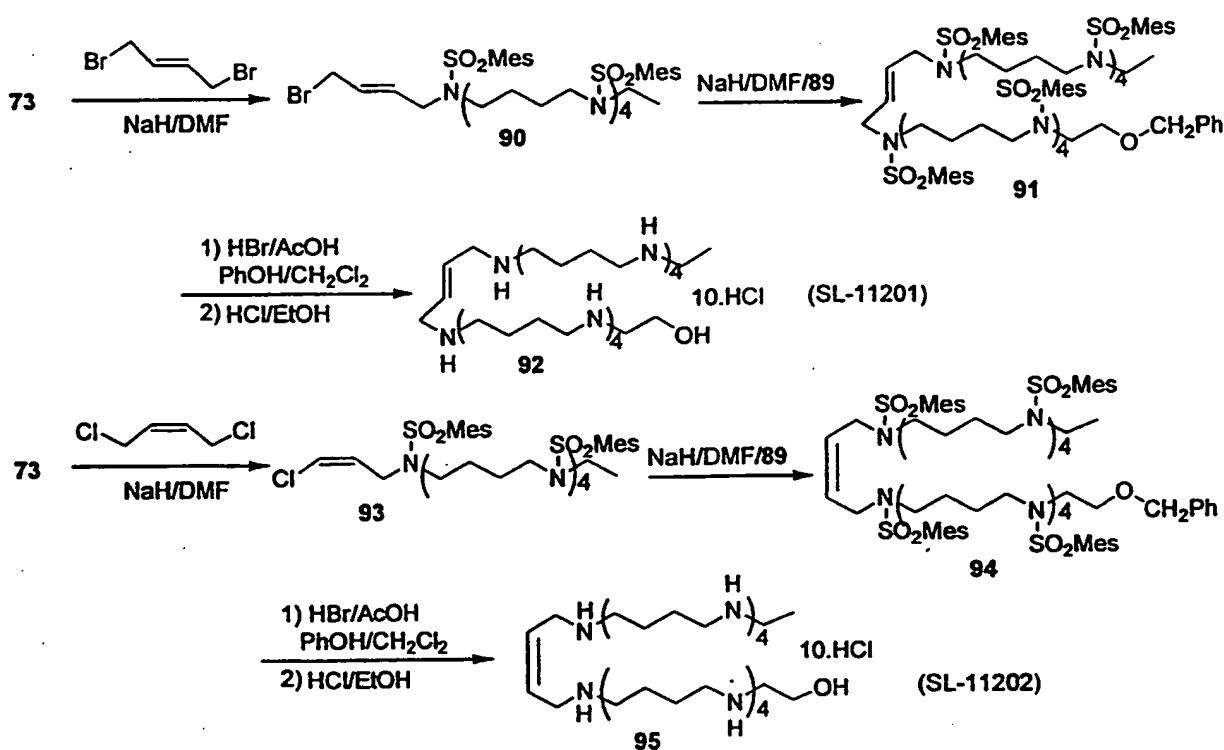
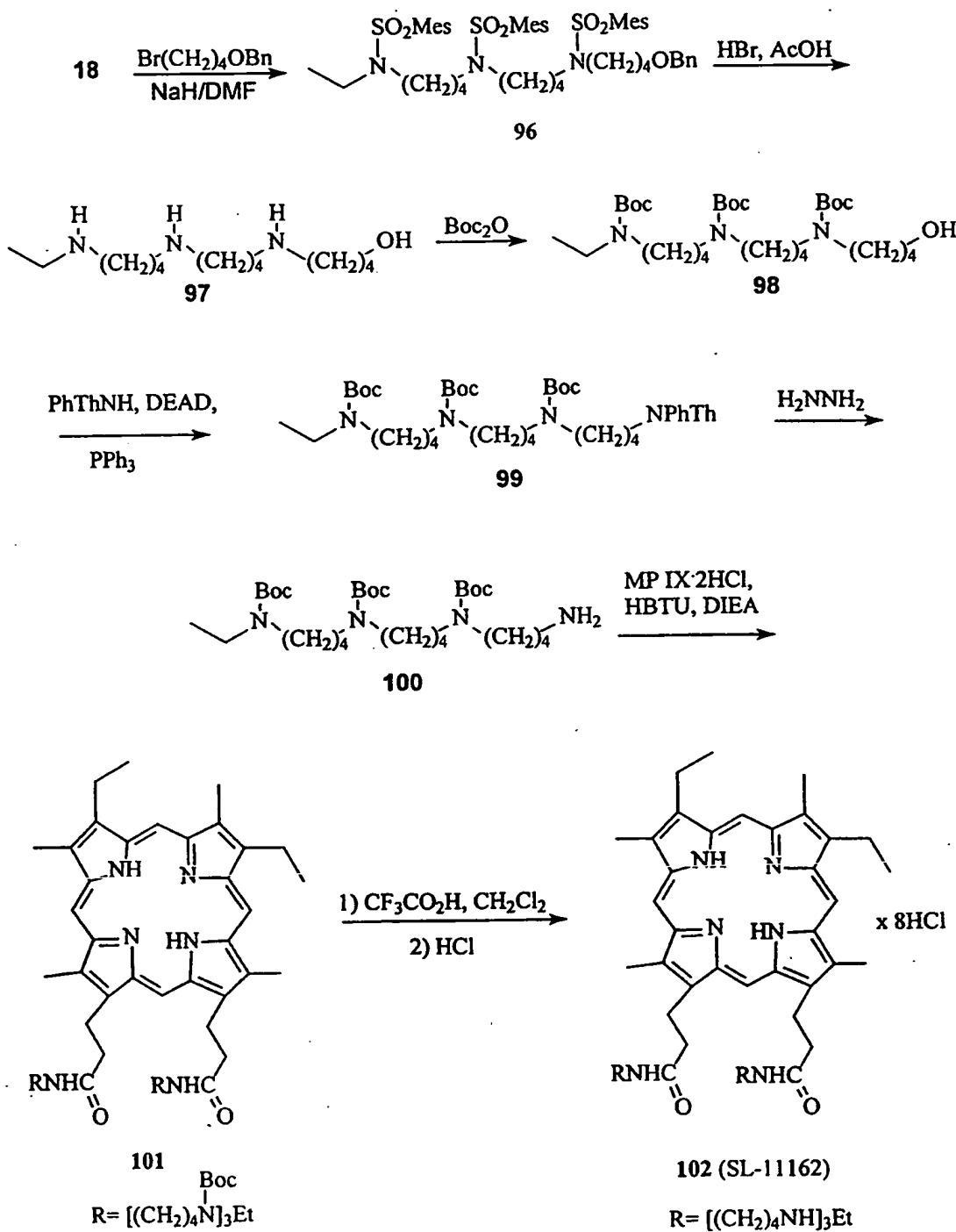


Figure 41

SCHEME 25



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FIGURE 42

SCHEME 26

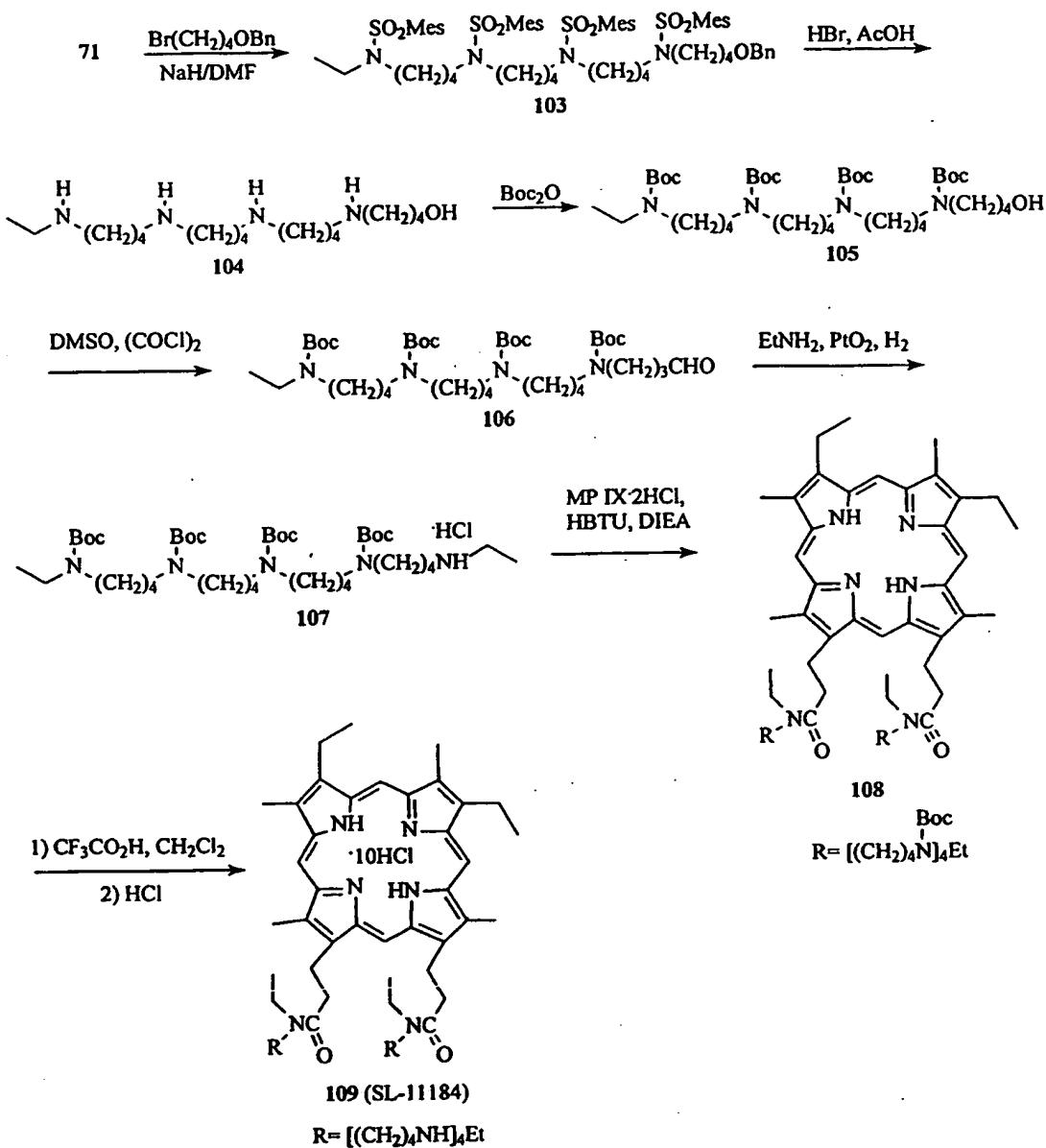


FIGURE 43

SCHEME 27

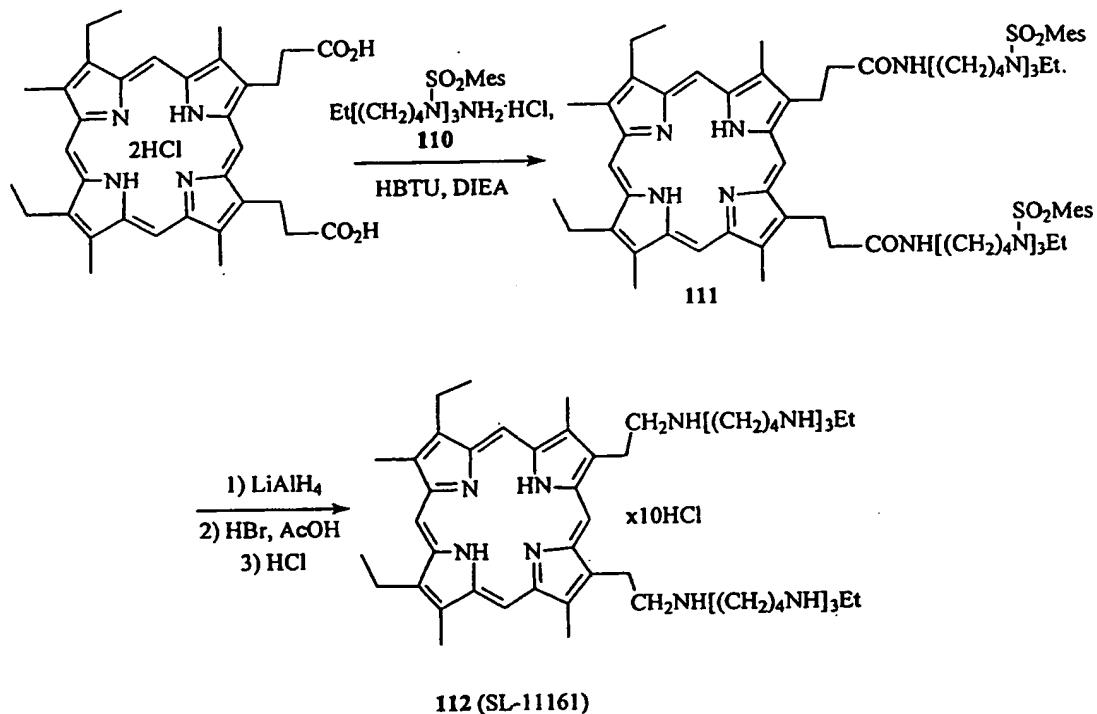
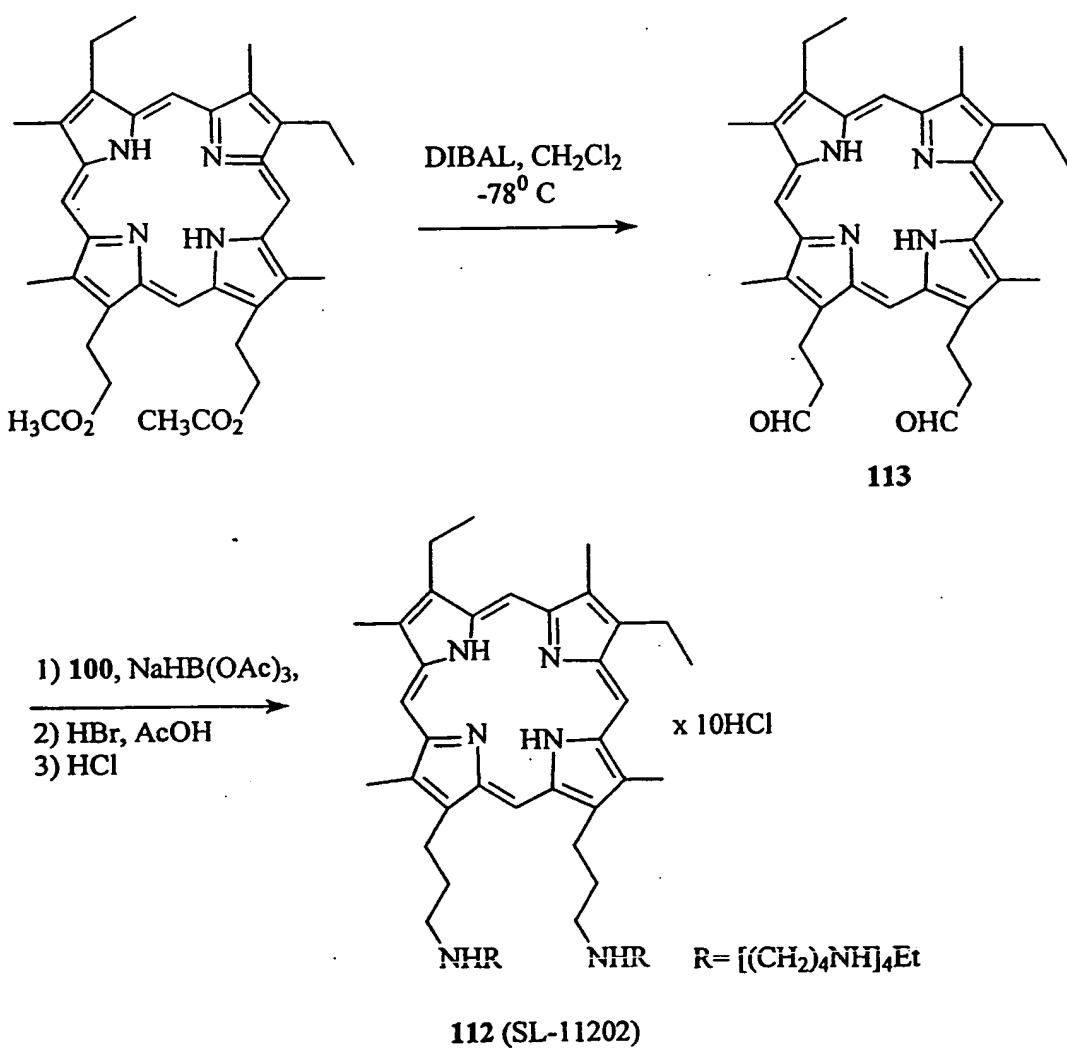
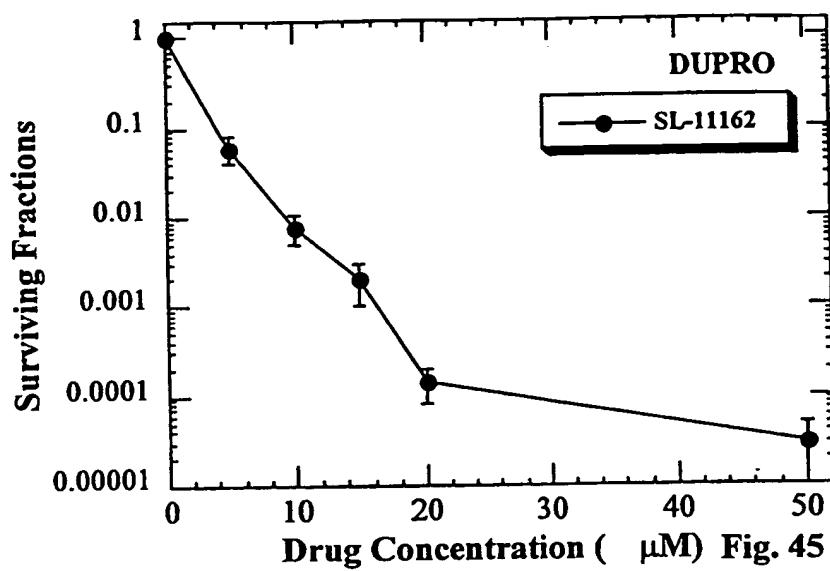
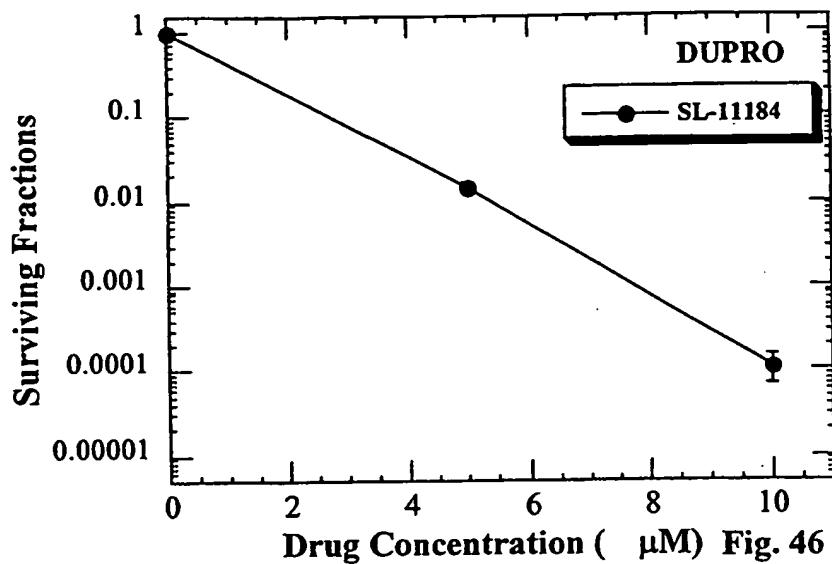


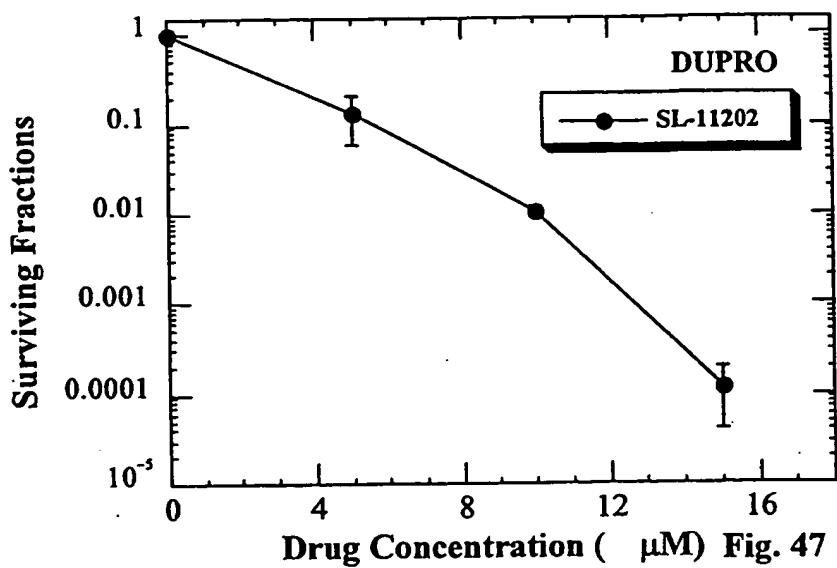
FIGURE 44

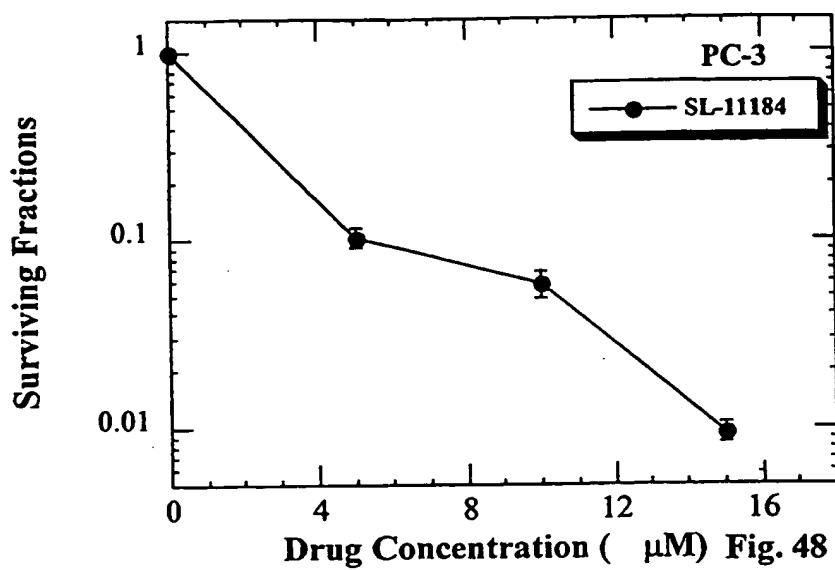
SCHEME 28

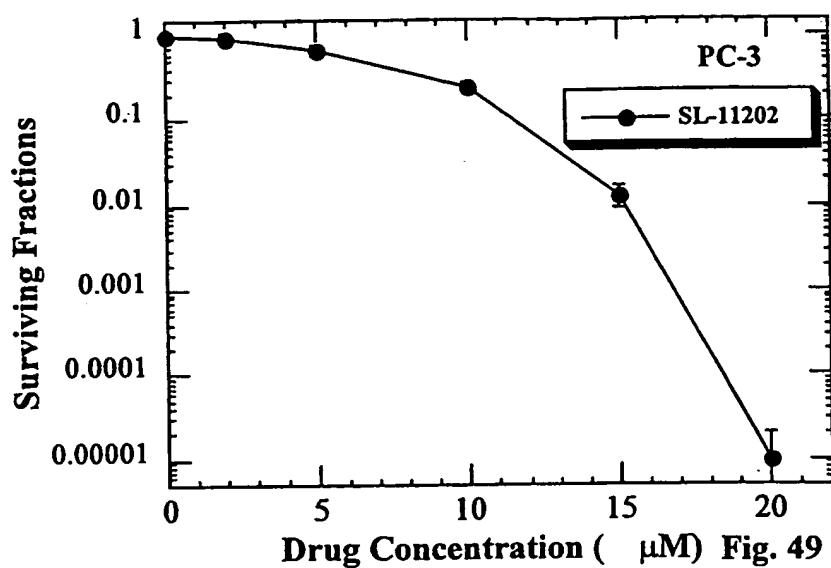












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35/00, A61K 31/155, 31/13, 31/40

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WI 53717 (US).

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(74) Agents: CERPA, Robert, K. et al.; Morrison & Foerster
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(54) Title: POLYAMINES AND THEIR USE IN THERAPY

(57) Abstract: Novel conformationally restricted polyamine analogs are provided, as well as compositions comprising these novel polyamine analogs. Methods of using the novel polyamine analogs in treatment of diseases such as cancer are also provided. Also provided is a method of delivering these analogs specifically to tumor cells by covalently attaching polyamine analogs to porphyrin compounds, along with novel polyamine-porphyrin covalent conjugates.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/11591

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D487/22 C07C211/22 C07C211/23 C07C211/18 C07C215/14
 C07C257/18 A61P35/00 A61K31/155 A61K31/13 A61K31/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

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IPC 7 C07D C07C A61P A61K

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PATENT ABSTRACTS OF JAPAN vol. 1996, no. 02, 29 February 1996 (1996-02-29) & JP 07 277964 A (NIPPON KAYAKU CO LTD), 24 October 1995 (1995-10-24) abstract	1-4, 16-23
Y	WO 95 18091 A (CIBA GEIGY AG ;FREI JOERG (CH); STANEK JAROSLAV (CH)) 6 July 1995 (1995-07-06) page 31 -page 49; examples 1-23	1-4, 16-23
Y	US 5 541 230 A (BASU HIRAK S ET AL) 30 July 1996 (1996-07-30) column 6, line 30 -column 20, line 64	1-4, 16-23

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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 02027 A (TEVA PHARMA ;GILAD GAD (IL); GILAD VARDA (IL); STERLING JEFFREY (I) 23 January 1997 (1997-01-23) page 44 —	13,17,18
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P,X	US 5 962 533 A (BERGERON JR RAYMOND J) 5 October 1999 (1999-10-05) page 7 -page 14; tables 1,2 —	13,16-23
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Int'l. Application No
PCT/US 00/11591

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Int'l Application No

PCT/US 00/11591

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/11591

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 17-23,31 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

1-4,7,8,11-14,16-31 (therof 16-23 and 31 only partially)
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-4 (completely), 16-23 (in part)

A compound according to the definition of claim 1.

2. Claims: 5,6 (completely), 16-23 (in part)

A compound according to the definition of claim 5.

3. Claims: 7,8,13,14 (completely), 16-23 (in part)

A compound according to definition of claim 7 or according to the definition of claim 13.

4. Claims: 9,10 (completely), 16-23 (in part)

A compound according to definition of claim 9.

5. Claims: 11,12 (completely), 16-23 (in part)

A compound according to the definition of claim 11.

6. Claims: 15; 16-23 (in part)

A compound according to the definition of claim 15.

7. Claims: 24-31

A porphyrin-polyamine conjugate and it's use.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/11591

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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INTERNATIONAL SEARCH REPORT

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Int'l Application No

PCT/US 00/11591

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